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N_2O
in the Atlantic Ocean and the Baltic Sea

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Sylvia Walter

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Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit selbständig und ohne unerlaubte Hilfen erstellt habe. Ferner habe ich weder diese noch eine ähnliche Arbeit an einer anderen Abteilung oder Hochschule im Rahmen eines Prüfungsverfahrens vorgelegt, veröffentlicht oder zur Veröffentlichung vorgelegt.

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Sylvia Walter took the samples and made most of the measurements, evaluated the data and wrote the manuscript. Ulrich Breitenbach supported the measurements. Hermann W. Bange and Douglas W.R. Wallace assisted with input to the manuscript and revision.

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Sylvia Walter designed the experiments, made the measurements, evaluated the data and wrote the manuscript, reviewed by Hermann W. Bange.

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Die vier Menschen, die ich am meisten liebe und denen
ich alles zu verdanken habe!

Aim of this study was the investigation of the vertical distribution of N_2O and the factors influencing this distribution, particularly in the North Atlantic Ocean and the Baltic Sea. Dissolved and atmospheric N_2O was measured during several cruises. These data, in combination with physical and chemical parameters, were used to determine sources and sinks of N_2O. Furthermore, possible production pathways of N_2O were investigated using the relationship between N_2O and relevant production parameters, and their relation to physical processes in the oceans. The hypothesis whether N_2O concentrations increased by oceanic iron fertilization via enhanced substrate supply for microbial respiration, was tested during the European Iron Fertilization Experiment (EIFEX) in the subpolar South Atlantic. First investigations about the vertical structure of bacterial communities in the subtropical North Atlantic were started, with the intention to find correlations between the community structure of bacteria and the distribution of N_2O. This work presents a detailed picture of the distribution of N_2O in the North Atlantic Ocean and the Baltic Sea. In summary, this study adds 80 profiles (1482 data points) of the Atlantic Ocean, and 26 profiles (231 data points) of the Baltic Sea to the database.

Atlantic Ocean

We could show that the production of N_2O in the subpolar North Atlantic is negligible, whereas the tropical North Atlantic is a net source of atmospheric N_2O, with a pronounced regional variability. The data indicate a production of N_2O by nitrification. However, the contribution of autotrophic nitrifiers to the bacterial community seems to be low, therefore a production of N_2O by others than autotrophic nitrifiers should be taken into account. In contrast to other authors the hypothesis of additional N_2O production triggered during Fe fertilization could not be confirmed.

Baltic Sea

N_2O in the Baltic Sea was measured after a major inflow of North Sea water. We could show that the inflow event has had a strong influence on the production and conversion of N_2O. The inflow led to a stimulation of the production of N_2O mainly by nitrification, but due to the clear water column stratification the Baltic Sea is not expected to be a source of atmospheric N_2O.

Ziel dieser Arbeit war die Untersuchung der vertikalen Verteilung von N_2O und den Faktoren, die diese Verteilung beeinflussen, insbesondere im Atlantik und der Ostsee. Auf mehreren Fahrten wurden sowohl atmosphärische als auch gelöste N_2O-Konzentrationen gemessen. Mit Hilfe dieser Daten, unterstützt durch physikalische und chemische Messdaten, wurden Quellen und Senken für N_2O bestimmt. Weiterhin wurden mögliche Produktionswege von N_2O mittels Korrelationsanalysen zwischen N_2O und produktionsrelevanten Parametern sowie deren Beziehung zu physikalischen Prozessen untersucht. Die Hypothese, ob eine erhöhte Biomasse-Produktion während einer Fe-Düngung zu einer erhöhten N_2O-Produktion führt, war Ziel der Untersuchungen während des European Iron Fertilization Experiments (EIFEX) im subpolaren Südatlantik. Außerdem wurden erste Untersuchungen zur vertikalen Struktur von Bakteriengemeinschaften im subtropischen Atlantik durchgeführt, um mögliche Korrelationen zwischen der Gemeinschaftsstruktur und den gemessenen N_2O-Konzentrationen zu finden. Die im Rahmen dieser Arbeit durchgeführten Studien liefern ein detailliertes Bild über die Vertikalstruktur der N_2O-Verteilung im Nordatlantik und der Ostsee. Im Atlantik sind somit 80 weitere N_2O-Tiefenprofile (1482 Datenpunkte) verfügbar, in der Ostsee weitere 26 (231 Datenpunkte).

Atlantischer Ozean

Wir konnten zeigen, dass die Produktion von N_2O im subpolaren Nordatlantik zu vernachlässigen ist, wohingegen der tropische Nordatlantik eine Quelle für atmosphärisches N_2O mit einer ausgeprägten regionalen Variabilität darstellt. Die Daten deuten auf eine N_2O-Produktion durch nitrifizierende Prozesse. Allerdings scheint der Anteil an autotrophen Nitrifizierern innerhalb der untersuchten Bakteriengemeinschaften gering zu sein, daher sollte eine N_2O-Produktion durch andere Organismen in Betracht gezogen werden. Die Hypothese einer erhöhten N_2O-Produktion ausgelöst durch Fe-Düngung konnten wir im Gegensatz zu anderen Autoren nicht bestätigen.

Ostsee

N_2O-Konzentrationen in der Ostsee wurden nach einem starken Nordseewassereinstrom gemessen. Wir konnten zeigen, dass dieser Einstrom einen starken Einfluss auf die Produktion und Umsetzung von N_2O hatte. Der Einstrom führte zu einer Stimulierung der N_2O-Produktion, wobei hauptsächlich nitrifizierende Prozesse eine Rolle spielen dürften. Aufgrund der deutlichen Stratifizierung der Ostsee wird diese nicht als Quelle für atmosphärisches N_2O angenommen.

Chapter 1

Introduction and thesis outline

Nitrogen and its derivatives are essential for life on earth; the nitrogen cycle is one of the major biogeochemical cycles. Due to the plethora of involved compounds, organisms, and different ecosystems the nitrogen cycle is extremely complex. The increasing anthropogenic influence on the nitrogen cycle, especially due to intensifying agriculture and its negative consequences by eutrophication and over-fertilization [*Prather et al.*, 2001], results in an increasing scientific interest. Especially the production and conversion of climatic relevant trace gases such as nitrous oxide (N_2O) receives more and more interest. Since the industrial revolution the amounts of these trace gases in the atmosphere increased continuously [*Prather et al.*, 2001]. Particularly agriculture activities contribute to enhanced N_2O emissions directly by emissions from agriculture soils and animal production and indirectly from agriculture nitrogen input resulting in the stimulation of N_2O producing processes in both terrestrial and aquatic ecosystems [*Mosier et al.*, 1998].

1. Nitrous oxide (N_2O)

1.1 Climatic relevance of N_2O

N_2O is the most abundant combined nitrogen compound in the atmosphere [*Jacobson et al.*, 2000]. The first evidence of N_2O in the atmosphere was shown in 1938, but not until 1970 the importance of N_2O for the chemistry of the atmosphere was discovered by Paul Crutzen [*Crutzen*, 1970; *McElroy and McConnel*, 1971]. N_2O influences directly and indirectly the Earth's climate: In the troposphere (0 – 12 km) it acts as a greenhouse gas like carbon dioxide (CO_2), directly contributing to global warming. Moreover, N_2O can reach up to the stratosphere (12 – 80 km) due to a relatively long atmospheric lifetime of 114 years [*Prather et al.*, 2001]. In the stratosphere it acts as the major source for nitric oxide radicals (NO) which are involved in one of the main ozone (O_3) reaction cycles [*Crutzen and Schmailzl*, 1983; *WMO*, 2003]. Since the industrial revolution at the end of the 19th century, the concentration of N_2O in the atmosphere has increased rapidly by about 17 %, from 270 ppb to 318 ppb in 2002 [*Sowers*, 2001; *Walter et al.*, 2004]. At present an annual increase of N_2O of 0.8 ppb is observed [*Prather et al.*, 2001].

1.2 Biological production of N_2O

Within the nitrogen cycle N_2O is an intermediary or a by-product of several biological processes. Nitrification and denitrification are commonly accepted to be the main N_2O producing processes [*Bonin et al.*, 2002; *Bouwman et al.*, 1995; *Kroeze et al.,* 1999] (see Fig. 1). Many organisms like bacteria, archaea, and even some fungi are able to produce N_2O via these two processes [*Cabello et al.*, 2004; *Könneke et al.*, 2005; *Zumft*, 1997; *Bleakley and Tiedje*, 1982]. Plants were also shown to be N_2O producers [*Smart and Bloom,* 2001; *Pihlatie et al.*, 2005]. However, nitrifiers are also able to denitrify, methanotrophic bacteria can nitrify, and even denitrification reactions are not strictly oxygen inhibited [*Wrage et al.*, 2001; *Sorokin et al.*, 2001]. Nitrification and denitrification are therefore no longer considered as processes specific only for single species or groups that are traditionally called nitrifiers and denitrifiers, thus the classical distinction between nitrifiers and denitrifiers begins to fade [*Wrage et al.*, 2001; *Zehr and Ward*, 2002]. Although nitrification and denitrification are assumed to be the most important production pathways, up to now the contribution of these processes to the global N_2O budget remains unclear and is discussed controversially [*Kroeze and Seitzinger*, 1998; *Codispoti et al.*, 2001; *Popp et al.*, 2002; *Yamagishi et al.*, 2005].

Fig.1 shows possible production pathways of N_2O during nitrification and denitrification.

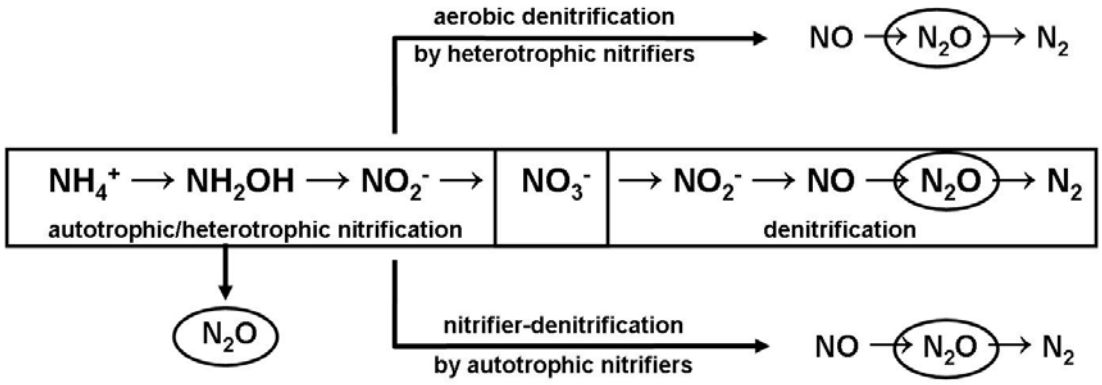

Fig. 1: Possible production pathways of N_2O during nitrification and denitrification

Although the processes have identical educts and products, they differ by the structure of used enzymes [*Moreno-Vivián et al.*, 1999; *Richardson and Watmough*, 1999]. Many organisms are able to use different enzymes for similar reactions or to switch between these pathways depending on the environmental conditions [*Moir et al.*, 1995; *Richardson*, 2000; *Wrage et al.*, 2001]. The yield of N_2O depends on the biosynthetic pathway,

the involved species, and the environmental conditions, e.g. the concentration of dissolved oxygen [*Abou Seada and Ottow*, 1985; *Jiang and Bakken*, 2000; *Brettar and Rheinheimer*, 1991; *Goreau et al.*, 1980; *Vollack and Zumft*, 2001; *Wetzel*, 1983]. The next sections give a short overview of the main N_2O producing processes.

1.2.1 Nitrification

In general, nitrification is an aerobic process in which organic or inorganic nitrogen compounds are oxidized to nitrate (NO_3^-) via hydroxylamine (NH_2OH) and nitrite (NO_2^-) [*Papen et al.*, 1989; *Schmidt et al.*, 1999] (see Fig. 1). In this process N_2O is considered as a by-product, produced either by a reduction of NO_2^- with NH_2OH [*Chalk and Smith*, 1983; *Ritchie and Nicholas*, 1972] or incomplete oxidation of NH_2OH [*Hooper and Terry*, 1979]. However, the exact metabolism is still under discussion [*Hynes and Knowles*, 1984; *Ostrom et al.*, 2000].

Nitrification can be conducted by both autotrophic and heterotrophic organisms. Autotrophic nitrifiers gain energy for CO_2 fixation by oxidation of ammonium (NH_4^+). Typically two groups of microorganisms are involved: NH_4^+-oxidizing bacteria (primary nitrifiers) and NO_2^--oxidizing bacteria (secondary nitrifiers) [*Bock et al.*, 1986]. Both groups of organisms were formerly classified as nitrifying bacteria in the family Nitrobacteraceae [*Buchanan*, 1917], but phylogenetically they are not closely related to each other [*Woese et al.*, 1984a; *Woese et al.*, 1984b; *Woese et al.*, 1985]. Several autotrophic organisms, namely NH_4^+-oxidizers are able to combine the nitrification pathway with denitrifying reactions under aerobic conditions, the so called nitrifier denitrification. Nitrifier denitrification includes the oxidation of NH_4^+ to NO_2^-, followed by the reduction of NO_2^- via N_2O to N_2 [*Wrage et al.*, 2001].

Besides autotrophic nitrifiers also heterotrophic nitrifiers are known. In contrast to the former group heterotrophic nitrifiers gain no energy during this process [*Robertson et al.*, 1988; *Schmidt et al.*, 1999; *Prosser*, 1989]. A possible advantage of this pathway might be the conservation of NO_3^- or NO_2^- for use during oxygen depleted conditions [*Castignetti and Hollocher*, 1984]. Similar to autotrophic nitrifiers they are also able to perform denitrifying reactions under aerobic conditions, with N_2O as an intermediate (see Fig. 1). Performed by heterotrophic nitrifiers this process is called aerobic denitrification [*Wrage et al.*, 2001]. Commonly the relevance of heterotrophic nitrifiers within the nitrogen cycle was considered to be low, due to their lower nitrification rates in compari-

son to autotrophic nitrifiers [*Knowles*, 1985; *Jetten et al.*, 1997]. However, in recent years much attention has been paid to heterotrophic nitrifiers because many of them are found to denitrify their nitrification products simultaneously [*Robertson et al.*, 1988; *Nishio et al.*, 1998]. This might lead to a significant underestimation of their nitrification ability and the release of N_2O by heterotrophic nitrifiers. *Papen et al.* [1989] found heterotrophic nitrifiers producing much more N_2O per cell than autotrophic nitrifiers. Thus, since heterotrophic nitrifiers are more widespread and include a variety of species [*Mevel and Prieur*, 2000], they may contribute significantly to the global N_2O budget [*Jetten et al.*, 1997].

1.2.2 Denitrification

Denitrification is the reduction of NO_3^- to N_2, with N_2O as a regular intermediate product [*Naqvi et al.*, 2000]. These reactions are characteristic for various subclasses of the Proteobacteria, but also organisms of other eubacterial and archaean genera are able to denitrify [*Chen et al.*, 2002; *Shapleigh*, 2000; *Cabello et al.*, 2004]. Denitrifiers are facultative anaerobic [*Schlegel*, 1992], and the change between aerobic and anaerobic metabolism is probably controlled particularly by the O_2 concentration, although little is known about the detailed mechanisms and signals [*Baumann et al.*, 1996; *John*, 1977; *Soerensen*, 1987]. Denitrifying enzymes are known to be O_2 sensitive, e.g., the O_2 induced inhibition of a N_2O reductase is stronger than that of other reductases [*Knowles*, 1982]. In suboxic habitats the inhibition of N_2O reductases therefore results in an accumulation of N_2O. In anoxic habitats, N_2O is used as an electron acceptor and is thereby reduced to N_2 [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978].

1.3 Sources and sinks of N_2O

An estimation of the quantity of nitrous oxide sources was published by the Intergovernmental Panel on Climate Change in 2001 [*Prather et al.* 2001] (see Fig. 2). N_2O is released by both anthropogenic and natural sources. Anthropogenic sources are particularly agricultural activities such as crop and stock farming, but also industrial production e.g. of nylon or picric acid. The dominating natural sources for N_2O are soils and oceans.

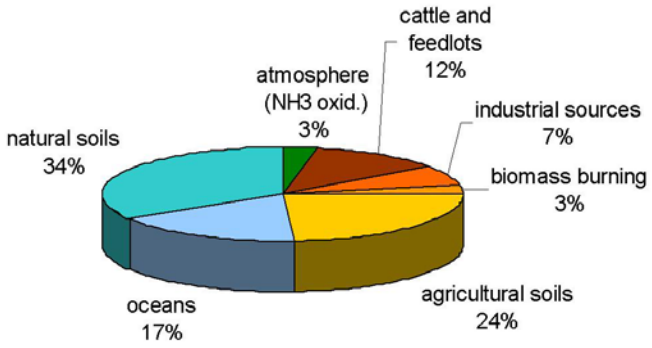

Fig. 2: Sources of atmospheric N_2O, modified from the IPCC report of 2001

N_2O is relatively stable. In the atmosphere degradation of N_2O takes place by photolysis and oxidative reactions [*McElroy et al.*, 1976; *Crutzen*, 1974]. Sinks of dissolved N_2O in the oceans are regions with depleted O_2 concentrations, where N_2O is used as an electron acceptor instead of O_2 in microbial processes [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978]. Oceanic sinks for atmospheric N_2O are regions of deep water formation [*Bange and Andreae*, 1999].

1.4 N_2O and Oceans

Oceans cover more than 70 % of the earth surface. They play an important role in biogeochemical cycles, particularly in conversion of compounds, their transport and the exchange with the atmosphere. One important function of the oceans is that they can be sinks or sources of atmospheric gases, e.g. trace gases such as CO_2 or N_2O. More than 25 % of naturally produced N_2O are emitted by the oceans, including continental shelves and estuaries [*Prather et al.*, 2001; *Seitzinger et al.*, 2000] (see Fig. 3). However, the contribution of different oceanic areas to the global N_2O budget is extremely variable. Particularly coastal regions, including estuarine and upwelling regions make a significant contribution to this budget [*Bange et al.*, 1996].

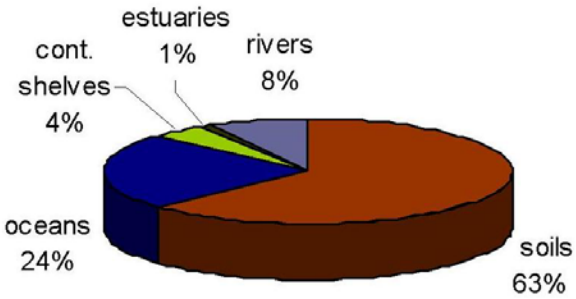

Fig. 3: Natural sources of atmospheric N_2O, modified from Seitzinger et al. 2000

Measurements of nitrification and denitrification rates in the ocean are sparse. To estimate the production of N_2O a linear regression between the excess of N_2O (ΔN_2O) and the apparent oxygen utilization (AOU) is commonly used [*Yoshinari*, 1976; *Cohen and Gordon*, 1978; *Butler et al.*, 1989; *Yoshida et al.*, 1989]. A positive correlation between these two parameters indicates nitrification, whereas in anoxic habitats (N_2O is used instead of O_2 as an electron acceptor) linear correlations between ΔN_2O and AOU are not existent [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978].

2. Study areas and short compilation of N_2O data

2.1 The Atlantic Ocean

The Atlantic Ocean (Fig. 4) is the second largest world's ocean, and extends into both the Arctic and Antarctic region. The Atlantic Ocean is divided by the Mid-Atlantic Ridge into a series of eastern and western basins. Together with several sills the Mid-Atlantic Ridge has a strong impact on the circulation of the deeper water layers. The Atlantic Ocean contains five mediterranean seas; amongst these the European Mediterranean Sea and the Baltic Sea. The most important feature is the formation of Deep Water in the northern part, which drives the oceanic water circulation, the so called "conveyer belt". Due to this constant renewal of water, driving also the input and the transport of atmospheric oxygen to the deep ocean, the Atlantic Ocean is well ventilated and permanently oxygenated. Therefore no permanent suboxic zones comparable to those found in the Arabian Sea or the eastern tropical Pacific Ocean exist [*Tomczak and Godfrey*, 2001]. Despite the fact that the Atlantic Ocean is by far the best investigated part of the world's ocean, data of the vertical N_2O distribution are com-

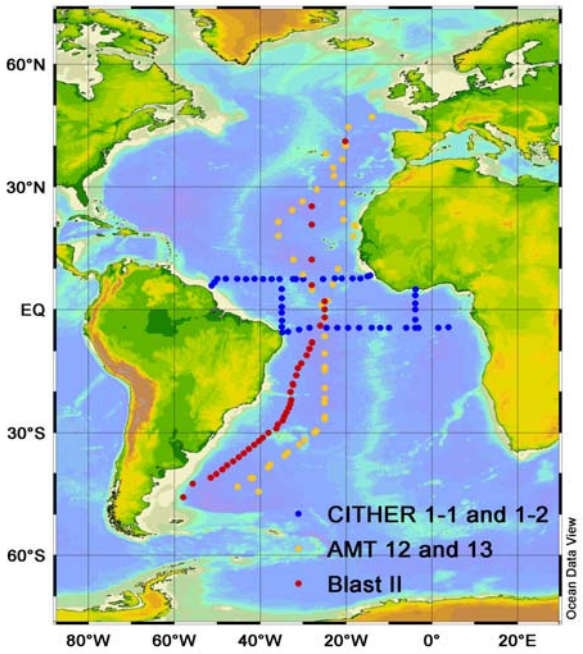

Fig. 4: Recent measurements of vertical N_2O profiles of the last 25 years.

parably sparse. In the last decades only a few vertical profiles of N_2O were published. Fig. 4 shows the locations of three cruises in the Atlantic Ocean. The cruises CITHER 1-1 and 1-2 and BLAST II measured N_2O concentrations down to the bottom, whereas data from AMT 12 and 13 provide N_2O concentrations down to approximately 300 m. A meridional transect along 30 °W and single stations in the subtropical and tropical northwestern Atlantic additionally have been measured. Table 1 gives an overview of the stations and data points measured in the Atlantic Ocean prior to our investigations.

Table 1: Overview of available N_2O data in the Atlantic Ocean, data points of *Junge and Hahn* [1971] were not available

region	cruise (year)	max. depth	number of stations / data points	reference
NE – SW Atlantic	BLAST II (1994)	5389 m	40 / 582	J. Butler, pers. communication [2005]
NE – SW Atlantic	AMT 12 (2003)	304 m	25 / 186	G. Forster, pers. communication [2005]
NE – SW Atlantic	AMT 13 (2003)	305 m	22 / 134	G. Forster, pers. communication [2005]
W – E tropical Atlantic	CITHER 1-1 (1993)	5678 m	21 / 440	[*Oudot et al.*, 2002]
W – E tropical Atlantic	CITHER 1-2 (1993)	3994 m	26 / 540	[*Oudot et al.*, 2002]
N - S Atlantic	1969/1970	3000 m	13 / n.a.	[*Junge and Hahn,* 1971]
NE Atlantic	1977/1978	2195 m	1 / 11	[*Cohen and Gordon*, 1979]
NW subtropical Atlantic	1976	~4800 m	14 / 248	[*Yoshinari*, 1976]
NW tropical Atlantic	1976	~1500 m	2 / 30	[*Yoshinari*, 1976]

In the present work data from four cruises in the Atlantic Ocean are reported. The first cruise (May / June 2002), started in Hamburg, Germany and followed the WOCE-A2 transect to Halifax, Canada. Depth profiles of N_2O in the subpolar North Atlantic were measured at 16 stations. The subtropical North Atlantic was investigated during March / April 2004. The cruise started in Fort de France, Martinique (French Antilles) in the western part of the Atlantic and ended in Lisbon (Portugal). Samples were taken at 37 stations. The tropical North Atlantic samples were taken during the M55-SOLAS cruise in October/November 2002. This cruise started in the western tropical North Atlantic in Willemstad, Curaçao (Netherlands Antilles) and followed a cruise track along 10 – 11 °N to Douala (Cameroon). The track included a transect to the equator between 26 and 23.5 °W. N_2O profiles were taken at 20 stations. The last cruise was during the European Iron Fertilisation Experiment (EIFEX) in the subpolar South Atlantic Ocean in February/March 2004. Here seven vertical N_2O profiles were measured, inside and outside of the iron fertilized patch.

2.2 The Baltic Sea

The Baltic Sea is a mediterranean sea of the Atlantic Ocean and part of the European continental shelf consisting of a series of basins (Arkona, Bornholm, and Gotland Basin; see Fig. 5). The basins show only a restricted horizontal and vertical water exchange due to shallow sills and a clear salinity stratification of water masses. A permanent halocline effectively prevents the vertical exchange and is the reason for the development of stagnant deep waters with oxygen depletion up to anoxia accompanied by accumulation of hydrogen sulphide (H_2S). Occasional inflow of oxygenated North Sea Water terminates these stagnation periods, however, during the last two decades these inflow events became less frequent and intense, thus the periods of anoxic conditions are increasing [*Tomczak and Godfrey*, 2001; *Nausch et al.*, 2003; *Feistel et al.*, 2003]. The most important recent inflow events during the last years were in 1993 and 2003 [*Feistel et al.*, 2003]. N_2O measurements in the Baltic Sea are as sparse as in the Atlantic Ocean. Only a few vertical profiles exist, mainly from three cruises Rönner made in spring and summer 1977 and 1980 [*Rönner*, 1983; *Rönner and Sörensson*, 1985]. He investigated the distribution of N_2O at 20 stations in the Baltic Proper, the Bothnian Sea and the Bothnian Bay. Additionally, two single profiles in the Baltic Proper (Gotland Deep and north of it) are available, measured in summer 1986 and 1987 by Brettar and Rheinheimer [*Brettar and Rheinheimer*, 1992]. In the present thesis one cruise in the Baltic Sea is reported, after the major inflow event in January 2003. In October 2003 N_2O concentrations were measured at 26 stations in the southern and central Baltic Sea.

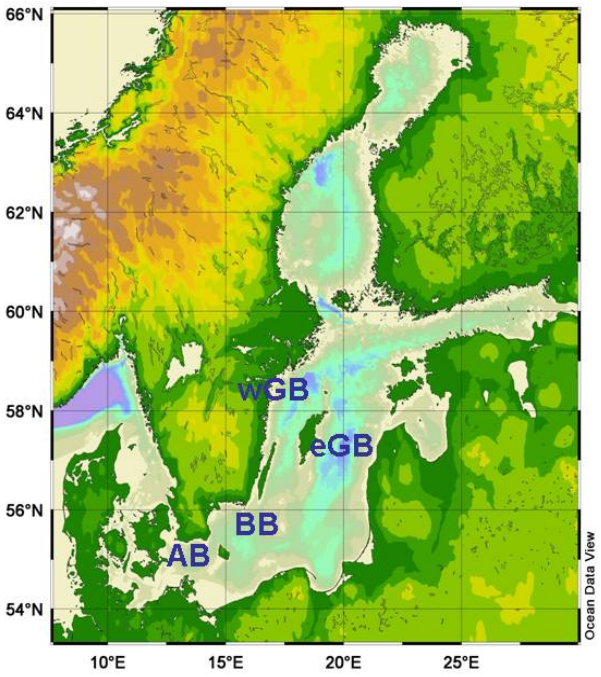

Fig. 5: The Baltic Sea; AB: Arkona Basin, BB: Bornholm Basin, eGB: eastern Gotland Basin, wGB: western Gotland Basin

3. Thesis outline

Since the industrial revolution the N_2O concentration in the atmosphere has increased due to human activities [*Kroeze and Seitzinger*, 1998], and because of the annual increasing rate N_2O might influence the climate of the next decades significantly [*Lashof and Ahuja*, 1990]. Oceans play an important role for the global budget of atmospheric N_2O emissions contributing more than a quarter of naturally produced atmospheric N_2O [*Seitzinger et al.*, 2000; *Prather et al.*, 2001].

Due to the insufficient number of vertical profiles of N_2O in the oceans, particularly the North Atlantic Ocean and the Baltic Sea, one aim of this study was to provide a more detailed and high resolution picture of the distribution of N_2O. These data should be used to investigate sources and sinks of N_2O, and to determine the role of the North Atlantic Ocean and the Baltic Sea as sources of atmospheric N_2O. Furthermore possible production pathways of N_2O should be investigated using the relationship between N_2O and relevant production parameters such as oxygen and nitrate, and their relation to physical processes in the oceans. During this thesis the possibility arose to start first investigations about the vertical structure of bacterial communities. 16S rRNA genes were used to obtain information of the vertical distribution of bacteria in the subtropical North Atlantic, with special regard to potential N_2O producers.

Chapter 2 gives an overview of the distribution of N_2O in the North Atlantic, whereas in chapter 3 the N_2O air-sea exchange in the tropical North Atlantic is presented. The possibility of stimulating N_2O production processes and counteracting the climatic benefits of a drawdown of atmospheric CO_2 during iron fertilization experiments in the South Atlantic is investigated in chapter 4. In chapter 5 the distribution of N_2O with regard to changed environmental conditions by a North Sea Water inflow in the Baltic Sea were studied. Since oceanic N_2O is mainly produced via microbial pathways we additionally investigated the bacterial community structures by 16S rRNA genes in the subtropical North Atlantic to obtain more information about the distribution of potential N_2O producers. This is content of chapter 6. Finally a short storage experiment is presented. Measurements are often not possible directly after sampling. For a validation of obtained data it is necessary to know, whether concentrations are affected over time between sample collections to measurements.

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Chapter 2

Nitrous oxide in the North Atlantic Ocean

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Abstract

In order to investigate the role of the North Atlantic Ocean as a source of atmospheric nitrous oxide and to decipher the major formation pathways of nitrous oxide, measurements of dissolved nitrous oxide were made during three cruises in the tropical, subtropical and subpolar North Atlantic in Oct. /Nov. 2002, Mar. /Apr. 2004, and May 2002, respectively. Nitrous oxide was close to equilibrium or slightly supersaturated in the surface layers suggesting that the North Atlantic acts as a weak source of nitrous oxide to the atmosphere. Depth profiles showed supersaturation throughout the water column with a distinct increasing trend from the subpolar to the tropical region. Lowest nitrous oxide concentrations, near equilibrium and with an average of 11.0 ± 1.7 nmol L^{-1}, were found in the subpolar North Atlantic where the profiles showed no clear maxima. Highest values up to 37.3 nmol L^{-1} occurred in the tropical North Atlantic with clear maxima at approximately 400 m. A positive correlation of nitrous oxide with nitrate, as well as excess nitrous oxide with AOU, was only observed in the subtropical and tropical regions. Therefore, we conclude that the formation of nitrous oxide occurs in the tropical region rather than in the subpolar region of the North Atlantic and suggest nitrification is the dominant formation pathway in the subtropical and tropical regions.

1. Introduction

Nitrous oxide (N_2O) is an important atmospheric trace gas due to its influence on the Earth's climate. In the troposphere N_2O acts as a greenhouse gas whereas in the stratosphere it is involved in the depletion of ozone by providing NO-radicals [*Prather et al.*, 2001]. Since the beginning of the industrial revolution the global mean tropospheric N_2O mole fraction has risen rapidly from 270 ppb up to 314 ppb in 1998 [*Prather et al.*, 2001]. About 24 % of the natural sources of atmospheric N_2O are contributed by the oceans [*Prather et al.*, 2001; *Seitzinger et al.*, 2000]. Nitrous oxide is an important component of the oceanic nitrogen cycle, mainly formed by the microbial processes of nitrification and denitrification [*Codispoti et al.*, 2001; *Goreau et al.*, 1980]: Nitrification is an aerobic two-step process in which ammonium is oxidized to nitrate ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$) by two different groups of bacteria. In this process nitrous oxide is assumed to be a by-product, however until now the exact pathway for N_2O production remains unclear. In suboxic habitats nitrate can be reduced by denitrification to molecular nitrogen ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), here nitrous oxide is an intermediate product. Especially at oxic/suboxic boundaries N_2O is produced by coupled nitrification and denitrification, due to the transfer of common intermediates [*Yoshinari et al.*, 1997]. Another possibility is aerobic denitrification, whereby under fully aerobic conditions organisms convert ammonia into nitrogen gas without the intermediary accumulation of nitrite [*Robertson et al.*, 1988]. All processes depend on oxygen concentrations, as well as the availability of substrates such as ammonium and nitrate. Many organisms are able to switch between different pathways depending on environmental conditions, and also the yield of N_2O during a process depends on environmental conditions [*Goreau et al.*, 1980; *Poth and Focht*, 1985; *Richardson*, 2000]. Positive correlations of N_2O with apparent oxygen utilization (AOU) or nitrate are interpreted as production of nitrous oxide by nitrification [*Yoshinari*, 1976; *Cohen and Gordon*, 1978; *Yoshida et al.*, 1989]. However, up to now the dominant production pathway for N_2O on the global scale and the contribution of different pathways still remains unclear [*Codispoti et al.*, 2001; *Popp et al.*, 2002].

Information on the vertical N_2O distribution in the North Atlantic is sparse, only a few profiles are available. The first vertical profiles for the North Atlantic were published by *Junge and Hahn* [1971] and *Yoshinari* [1976], additional data were collected by *Butler et*

al. [1995], and recently data from a transect at 7°30´ N were reported by *Oudot et al.* [2002]. In this paper we present a comprehensive set of 73 vertical profiles of nitrous oxide from three trans-Atlantic cruises, covering the subpolar North Atlantic, the subtropical and the tropical North Atlantic. Based on these new data, we examine the regional differences of the N_2O distribution and its formation pathways.

2. Study area

2.1 Research cruises

Samples from the three cruises were collected over the period from May 2002 to April 2004 (see Fig. 1).

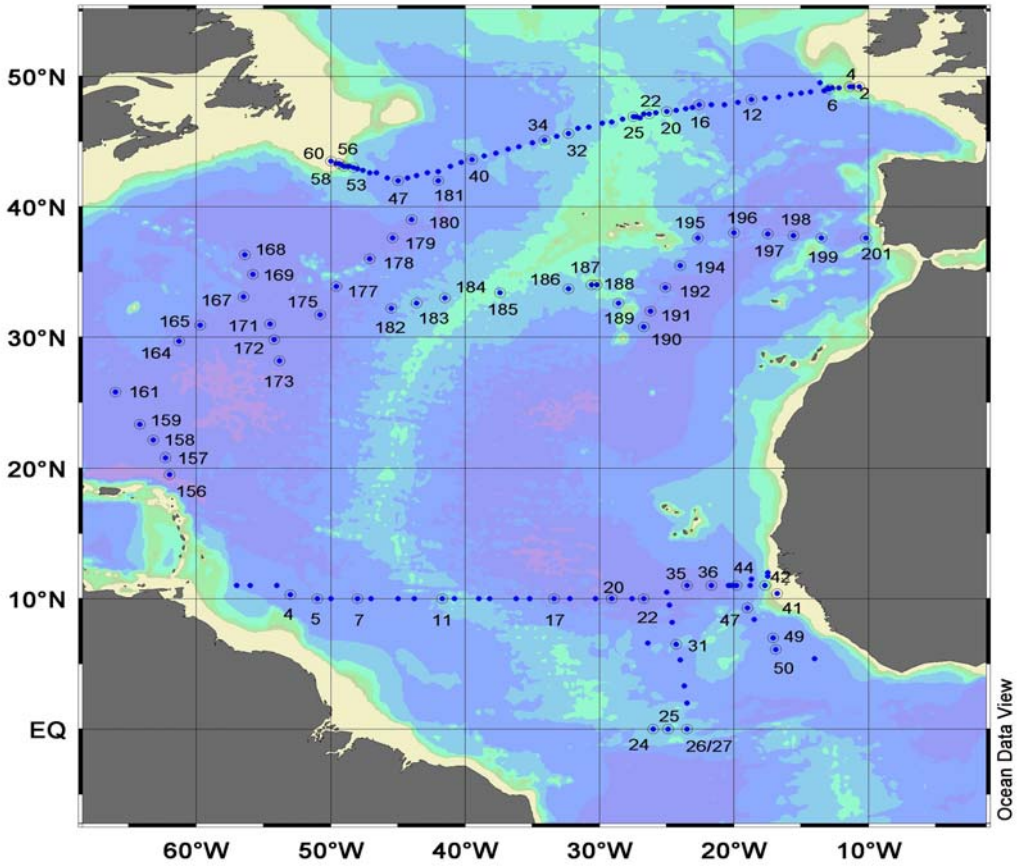

Fig. 1: Cruise tracks for `Gauss 384-1´ (subpolar North Atlantic, May 16th to June 14th 2002), `Meteor 60-5´ (subtropical North Atlantic, March 9th to April 14th 2004) and `Meteor 55´ (tropical North Atlantic, October 13th to November 16th 2002). Numbers are given for stations where N_2O profiles were measured.

The first cruise (May / June 2002), started in Hamburg, Germany with the German research vessel `Gauss´. The cruise track followed the WOCE-A2 transect to Halifax, Canada. Depth profiles of N_2O were measured at 16 stations. The WOCE-A2 transect is located between 42 °N and 49 °N.

The subtropical North Atlantic was investigated during March / April 2004 onboard the research vessel ´Meteor`. The cruise started in Fort de France, Martinique (French Antilles) in the western part of the Atlantic and ended in Lisbon (Portugal). Samples were taken at 37 stations. Most stations were co-located with stations where samples were taken during the Transient Tracers in the Ocean Program (TTO) in 1982.

The tropical North Atlantic samples were taken during the M55-SOLAS cruise [*Wallace and Bange*, 2004] in October/November 2002, again with the German research vessel ´Meteor`. This cruise started in the western tropical North Atlantic in Willemstad, Curaçao (Netherlands Antilles) and followed a cruise track along 10 – 11 °N to Douala (Cameroon). The track included a transect to the equator between 26 °W and 23.5 °W. N_2O profiles were taken at 20 stations.

2.2 Hydrography

Several water masses in the North Atlantic can be identified in the T-S-diagram based on data from the three cruises (see Fig. 2). The main Atlantic water masses were identified according to commonly used classification schemes [Tomczak, 1999; Alvarez et al., 2004; Aiken et al., 2000; Joyce et al., 2001; Poole and Tomczak, 1999].

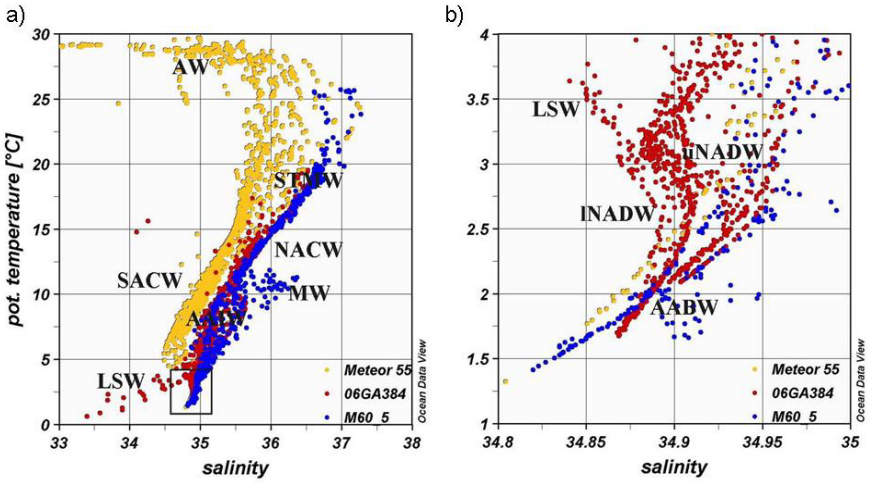

Fig. 2: T-S-diagram of the North Atlantic; 2a) T-S-diagram with data from all three cruises; 2b) T-S-diagram in Fig. 2a framed by box.; AW: Amazon Water; STMW: Subtropical Mode Water; MW: Mediterranean Water; SACW: South Atlantic Central Water; NACW: North Atlantic Central Water; ;AAIW: Antarctic Intermediate Water, AABW: Antarctic Bottom Water; lNADW: lower North Atlantic Deep Water; uNADW: upper North Atlantic Deep Water; LSW: Labrador Sea Water

The WOCE A2 transect (Gauss 384-1 cruise), is located at the boundary region between the subpolar gyre [*Gordon*, 1986] and the subtropical gyre [*Krauss*, 1996]. This region is highly variable, characterized by the exchange of upper-ocean water between the gyres mainly via the North Atlantic Current, and the Labrador Current. One of the most important water masses here is the Labrador Sea Water (LSW). These water masses provide the major part of the North Atlantic Intermediate Water in combination with the outflow of Mediterranean Sea Water (MW), which is detected in the eastern basin of the subtropical Atlantic Ocean near the Strait of Gibraltar [*Richardson et al.*, 2000] and the Antarctic Intermediate Water (AAIW) from the south [*Lorbacher*, 2000]. Additional water masses of the southern hemisphere that penetrate into the North Atlantic are the South Atlantic Central Water (SACW) and the Antarctic Bottom Water (AABW). SACW flows northwards, and mixes with the North Atlantic Central Water (NACW) at approximately 15 °N in the western and 20 °N in the eastern basin [*Poole and Tomczak*, 1999; *Aiken et al.*, 2000].

A typical freshwater influence was found during the Meteor 55 cruise in the western tropical North Atlantic. Water of the Amazon was detected in the surface water, identified by high temperatures and low salinity. These plumes of freshwater are transported northwards by the North Brazil Current and eastwards by the equatorial current system [*Fratantoni and Glickson*, 2002].

3. Material and methods

Water samples for N_2O analysis were collected in triplicate from various depths, taken with a 24-Niskin-bottle rosette, equipped with a CTD-sensor. The analytical method applied is a modification of the method described by [*Bange et al.*, 2001]. Bubble free samples were taken immediately following oxygen sampling in 24 mL glass vials, sealed directly with butyl rubber stoppers and crimped with aluminium caps. To prevent microbial activity, samples were poisoned with 500 µL of 2 mM mercury chloride solution. Then 10 mL of sample was replaced with a helium headspace for each vial, and the samples were allowed to equilibrate for at least two hours at room temperature (temperature was recorded continuously). A 9 mL subsample from the headspace was used to flush a 2 mL sample loop after passing through a moisture trap (filled with Sicapent®, Merck Germany). Gaschromatographic separation was performed at 190 °C on a packed molecular sieve column (6ft x 1/8"SS, 5A, mesh 80/100, Alltech GmbH, Germany). The N_2O was detected with an electron capture detector. A mixture of argon with 5 % by volume methane was used as carrier gas with a flow of 21 mL min^{-1}. For the two-point calibration procedure we used standard gas mixtures with 311.8 ± 0.2 ppb and 346.5 ± 0.2 ppb N_2O in synthetic air (Deuste Steininger GmbH, Mühlhausen Germany). The standard mixtures have been calibrated against the NOAA (National Oceanic and Atmospheric Administration, Boulder, Co.) standard scale in the laboratories of the Air Chemistry Division of the Max Planck Institute for Chemistry, Mainz, Germany.

Calculations

N_2O water concentrations (C_{N2O}) were calculated as follows:

$$C_{N2O}\left[\text{nmol L}^{-1}\right] = \left(\beta \times P \, V_{wp} + \frac{x \, P}{R \, T} V_{hs}\right) / V_{wp} \qquad (1)$$

where β stands for the Bunsen solubility in nmol L^{-1} atm^{-1} [*Weiss and Price*, 1980], x is the dry gas mole fraction of N_2O in the headspace in ppb, P is the atmospheric pressure in atm, V_{wp} and V_{hs} stand for the volumes of the water and headspace phases, respectively. R is the gas constant ($8.2054\ 10^{-2}$ L atm mol^{-1} K^{-1}) and T is the temperature during equilibration. The salinity was measured by the CTD-Sensor during water sample collection. The overall relative mean analytical error was estimated to be ± 1.8 %.

The excess N_2O (ΔN_2O) was calculated as the difference between the calculated N_2O equilibrium concentration and the measured concentration of N_2O as follows

$$\Delta N_2O\ [\text{nmol L}^{-1}] = N_2O\ (\text{observed}) - N_2O\ (\text{equilibrium}). \quad (2)$$

To calculate the N_2O equilibrium concentration we used three different atmospheric mole fractions. Between the mixed layer and the atmosphere, N_2O exchanges in about three weeks [*Najjar*, 1992], thus we calculated ΔN_2O in the mixed layer using the actual atmospheric N_2O value of 318 ppb measured during the 'Meteor 55' cruise [*Walter et al.*, 2004]. Below the thermocline, exchange with the atmosphere is unlikely, thus, calculated N_2O equilibrium concentrations depend on the atmospheric N_2O mole fraction at the time of deep-water formation. However, the exact atmospheric mole fraction of N_2O during deep-water formation is unknown because of uncertainty in age determination of water masses. Generally, tropical Atlantic deep waters below 2000 m seem to be older than 200 years [*Broecker and Peng*, 2000]. Therefore for depths > 2000 m ΔN_2O was calculated with the tropospheric preindustrial value of 270 ppb [*Flückiger et al.*, 1999]. An average of the actual and the preindustrial atmospheric value (i.e., 294 ppb) was used for the depth range between the upper thermocline and 2000 m. The thermocline was defined as the depth where the temperature differs from the surface temperature by more than 0.5 °C [*Tomczak and Godfrey*, 2001]. For the subtropical and subpolar region we calculated ΔN_2O with these same mole fractions, although the age of water masses is different to the tropical Atlantic and therefore some values may be underestimated, whereas others may be overestimated. The resulting uncertainties of ΔN_2O are about 10-15 %; however, our conclusions are not significantly affected by this uncertainty. The equilibrium values of dissolved oxygen (O_2) were calculated with the equation given by [*Weiss*, 1970].

The apparent oxygen utilization (AOU) was calculated as followed:

$$\text{AOU}\ [\mu\text{mol L}^{-1}] = O_2\ (\text{equilibrium}) - O_2\ (\text{observed}). \quad (3)$$

4. Results

4.1 Distribution of nitrous oxide in the North Atlantic

4.1.1 N_2O distribution along isopycnal levels

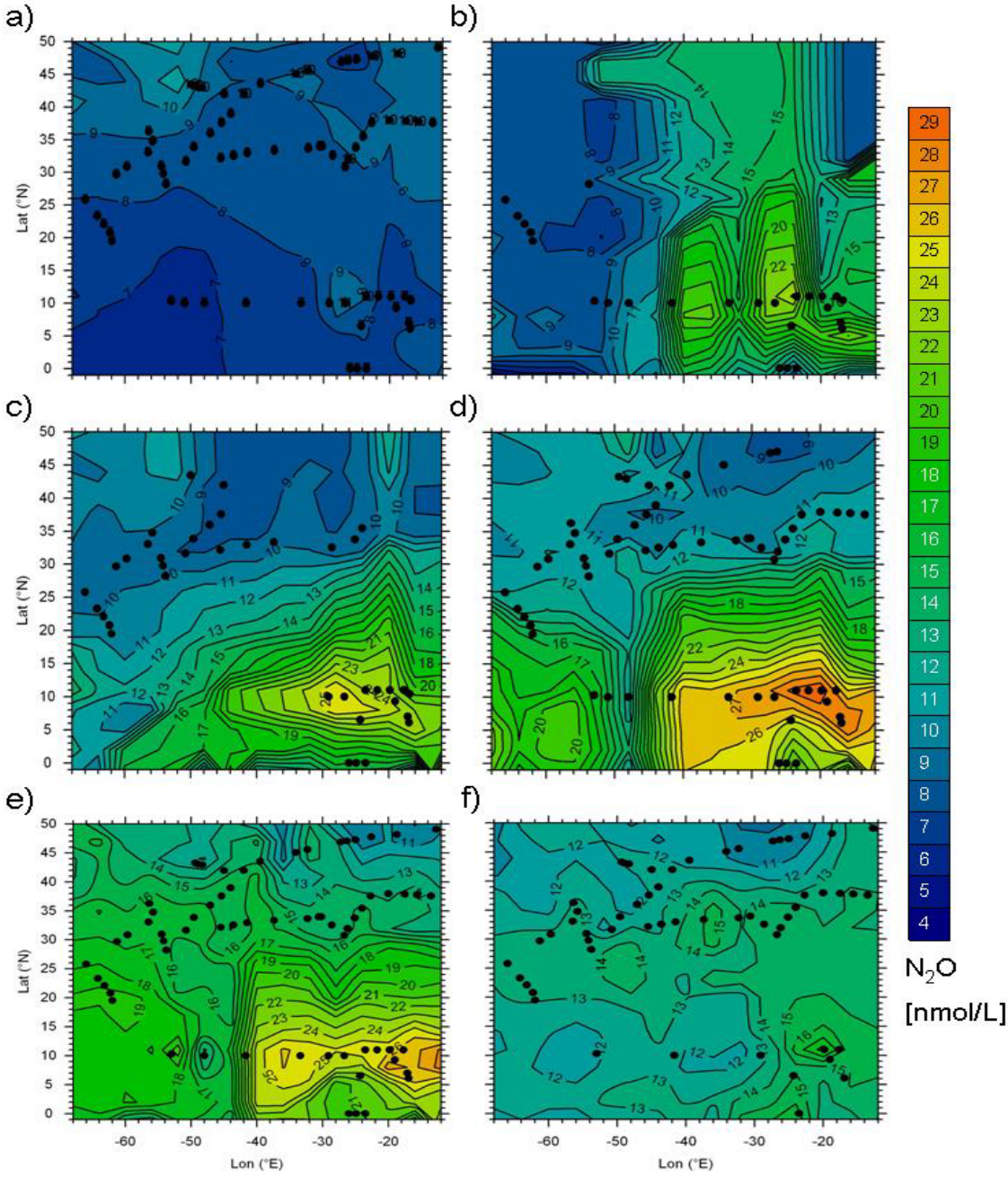

Fig. 3: Distribution of N_2O in the North Atlantic along isopycnal levels. Dots indicate stations with available data for the isopycnal levels. 3a) surface – thermocline; 3b) thermocline – 26.0; 3c) 26.1 – 26.5; 3d) 26.6 – 27.0; 3e) 27.1 – 27.5; 3f) 27.6 – 27.93

In the surface layer of the North Atlantic (Fig. 3a) N_2O concentrations were relatively uniform with 8.5 ± 1.2 nmol L^{-1}. In the region of the Labrador Current N_2O concentrations were enhanced with an average of 11.6 ± 0.9 nmol L^{-1}. During the 'Meteor 55' cruise, a plume of Amazon Water had been identified in the western basin of the tropical North Atlantic [*Körtzinger*, 2003]. In contrast to [*Oudot et al.*, 2002], who reported enhanced values in the plume of the Amazon River, we found no influence on N_2O concentrations [*Walter et al.*, 2004].

Below the thermocline, N_2O concentrations were variable with respect to depths and regions. We found highest concentrations in the eastern basin of the tropical North Atlantic throughout the water column, with maximum concentrations on σ_θ surfaces between 26.3 and 27.1 (Fig. 3b-e). At the Midatlantic Ridge, located at approximately 40 °W, a distinct boundary between the western and eastern Atlantic basins was observed (Fig. 3d-e). In the eastern subtropical North Atlantic, at approximately 1000 m (σ_θ 27.6-27.7), a tongue of outflow water from the Mediterranean Sea was detected by higher values of salinity and temperature [*Richardson et al.*, 2000]. However, we found no apparent influence of the Mediterranean water on N_2O concentrations.

Like the surface layer, deep waters (Fig. 3f) showed nearly uniform N_2O concentrations, though with higher values of 13.3 ± 1.6 nmol L^{-1}. However, a weak but distinct trend of decreasing concentrations from the tropics (13.1 ± 1.3 nmol L^{-1}) to the subpolar North Atlantic (11.1 ± 1.4 nmol L^{-1}) could be observed.

4.1.2 Vertical N_2O distribution

The vertical distribution of N_2O showed characteristically different profiles in different regions of the North Atlantic (Fig. 4a-c), and between the western and eastern basins of these regions (Fig. 5a-c).

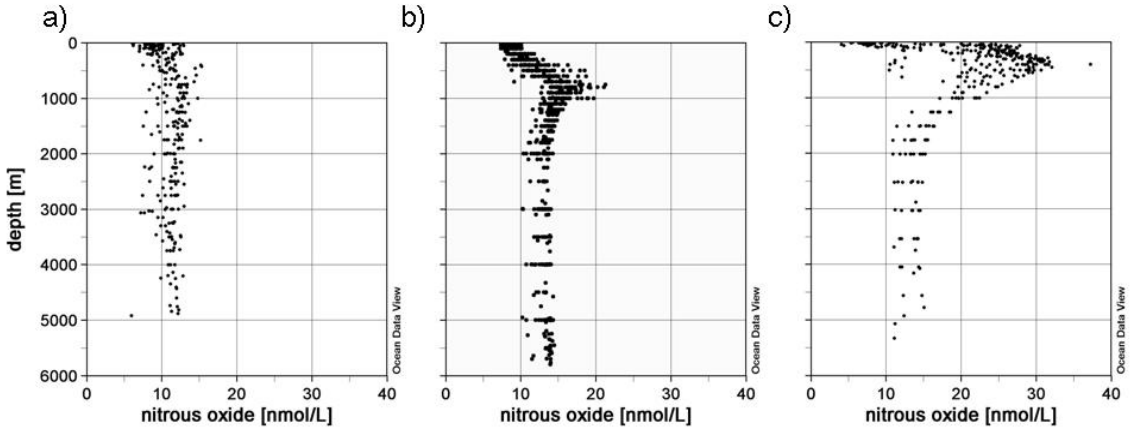

Fig. 4: N_2O concentration in the North Atlantic plotted against depth. 4a) subpolar, 4b) subtropical, 4c) tropical North Atlantic

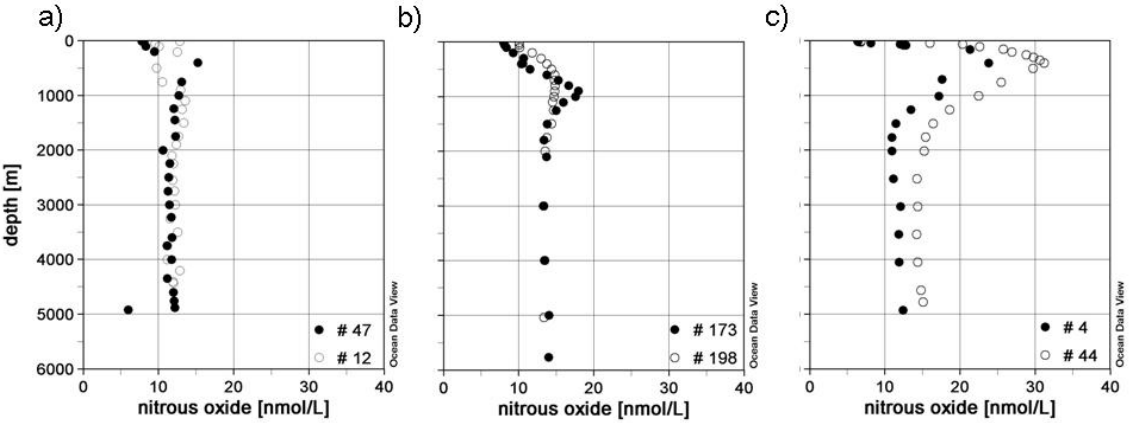

Fig. 5: Selected vertical N_2O profiles in the western basin (filled symbols) and the eastern basin (open symbols) in the North Atlantic. Stations were indicated by numbers. 5a) subpolar, 5b) subtropical, 5c) tropical North Atlantic

In the subpolar North Atlantic (Fig. 4a) vertical gradients of nitrous oxide were weak over the complete cruise track, with no clear or only a very weakly pronounced sub-surface maximum. N_2O concentrations were near equilibrium (11.0 ± 1.3 nmol L^{-1}) throughout the water column, with average concentrations of 8.6 ± 1.4 nmol L^{-1} in the surface layer (σ_θ 25.3-27.0) and 11.3 ± 1.5 nmol L^{-1} below the thermocline down to the bottom (σ_θ 26.2-27.7). No differences between the western and the eastern basin were found (Fig. 5a).

In contrast, N_2O distributions and profiles in both the subtropical and tropical North Atlantic showed strong variations with water depth (Fig. 4b-c, Fig. 5b-c). In both regions, the profiles generally had one distinct maximum. In the surface layer (σ_θ 19.3-26.8) concentrations were uniform, increasing below the thermocline up to a maximum and decreasing down to approximately 2000 m (σ_θ 22.1-27.8). Below 2000 m (σ_θ 27.8-27.9) N_2O concentrations were nearly constant with depth in both basins.

In the subtropical North Atlantic (Fig. 4b) N_2O surface concentrations were 8.7 ± 0.7 nmol L^{-1}, comparable to those in the subpolar North Atlantic. Maximum values were found at depths between 600 to 1000 m (σ_θ 26.7 – 27.7); values ranged from 14.0 in the eastern basin (#195) to 21.3 nmol L^{-1} in the western basin (#156). Below 2000 m ($\sigma_\theta > 27.8$), concentrations were nearly constant at 13.1 ± 0.9 nmol L^{-1}. Profiles in the western subtropical North Atlantic showed distinct maxima, while in the eastern basin no clear maximum was expressed (Fig. 5b). From the western to the eastern basin maximum concentrations decreased slightly from 17.7 ± 1.4 nmol L^{-1} to 15.1 ± 0.7 nmol L^{-1}. East of the Midatlantic Ridge maxima were not clearly expressed and were broader. Additionally, maximum ΔN_2O values were lower in the eastern (5.5 ± 0.6 nmol L^{-1}) than in the western basin (7.9 ± 1.3 nmol L^{-1}).

In the tropical North Atlantic (Fig. 4c) surface concentrations, with an average of 7.4 ± 1.1 nmol L^{-1}, were slightly lower than in the subtropical and subpolar North Atlantic. In contrast to the subtropical North Atlantic, maxima of N_2O concentrations were found at shallower depths of approximately 400m (σ_θ 26.8 – 27.1). The maximum values were higher in general, and ranged from 23.8 nmol L^{-1} in the western basin (#4) to 32.1 nmol L^{-1} in the eastern basin (#47). At station #36, located in the Guinea Dome area [*Siedler et al.*, 1992; *Snowden and Molinari*, 2003], we observed the highest N_2O values of about 37.3 nmol L^{-1} at 400 m (σ_θ 27.0) (Fig. 4c). At the equatorial stations the N_2O maxima were found at shallower water depths (240 m to 280 m, σ_θ 26.6 – 27.0). Maximum values ranged from 22.3 nmol L^{-1} (#26) to 24.9 nmol L^{-1} (#24). Below 2000 m ($\sigma_\theta > 27.8$) concentrations in the tropical North Atlantic were similar to those in the subtropics with an average of 13.2 ± 1.3 nmol L^{-1}. In both basins of the tropical North Atlantic profiles looked similar with sharp and clear maxima, however, concentrations throughout the water column increased from west to east (Fig. 5c). Below 2000 m ($\sigma_\theta > 27.8$) N_2O concentrations were about 2 nmol L^{-1} higher in the eastern than in the

western basin, whereas the difference of the maximum values was even higher (approximately 8 nmol L^{-1}).

N_2O profiles of the subtropical and tropical North Atlantic are in good agreement, both in absolute concentrations and shape of profiles, with those measured during the Bromine Latitudinal Air/Sea Transect II (BLAST II) cruise in Oct./Nov. 1994 [*Butler et al., 1995*] http://www.cmdl.noaa.gov/hats/ocean/blast2/blastii.html. Below 1500 m in the North Atlantic as far as 20 °S, the mean N_2O concentration observed by Butler et al. was about 13.5 ± 1.0 nmol L^{-1} (n = 18) which is in good agreement with our measurements (12.6 ± 1.5 nmol L^{-1}, n = 449).

4.2 Comparison of nitrous oxide with other parameters

Parameters most relevant for comparison with nitrous oxide are those assumed to be directly in connection with production pathways of N_2O, like oxygen or the apparent oxygen utilization (AOU), and nitrate. In general, we found ΔN_2O positively correlated with AOU and nitrate (Fig. 6). However, in view of differences between the basins, these correlations might not be sufficient and need higher resolution. Therefore data were divided as shown in Figure 7.

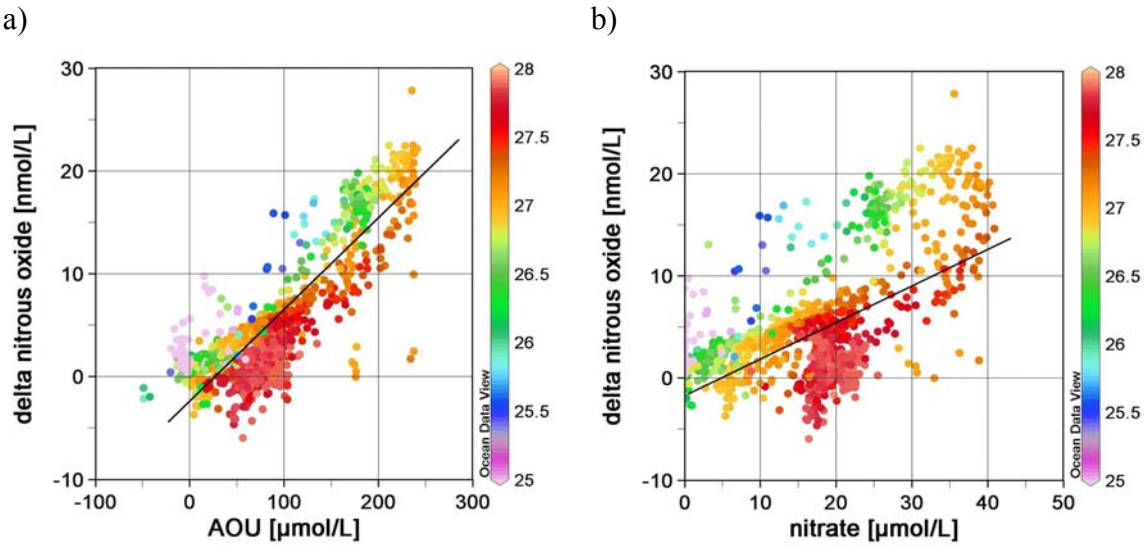

Fig. 6: ΔN_2O in comparison with AOU (6a) and nitrate (6b) for the North Atlantic, sigma σ_θ is colour coded in kg m^{-3}.
y (ΔN_2O) = 0.089x (AOU) – 3.245 R^2 = 0.71
y (ΔN_2O) = 0.331x (NO_3^-) – 1.263 R^2 = 0.32

In Fig. 7a-f correlations between the excess of N_2O (ΔN_2O) with AOU (Fig. 7a-c) and with NO_3^- (Fig. 7d-f) are divided for all data of the respective regions.

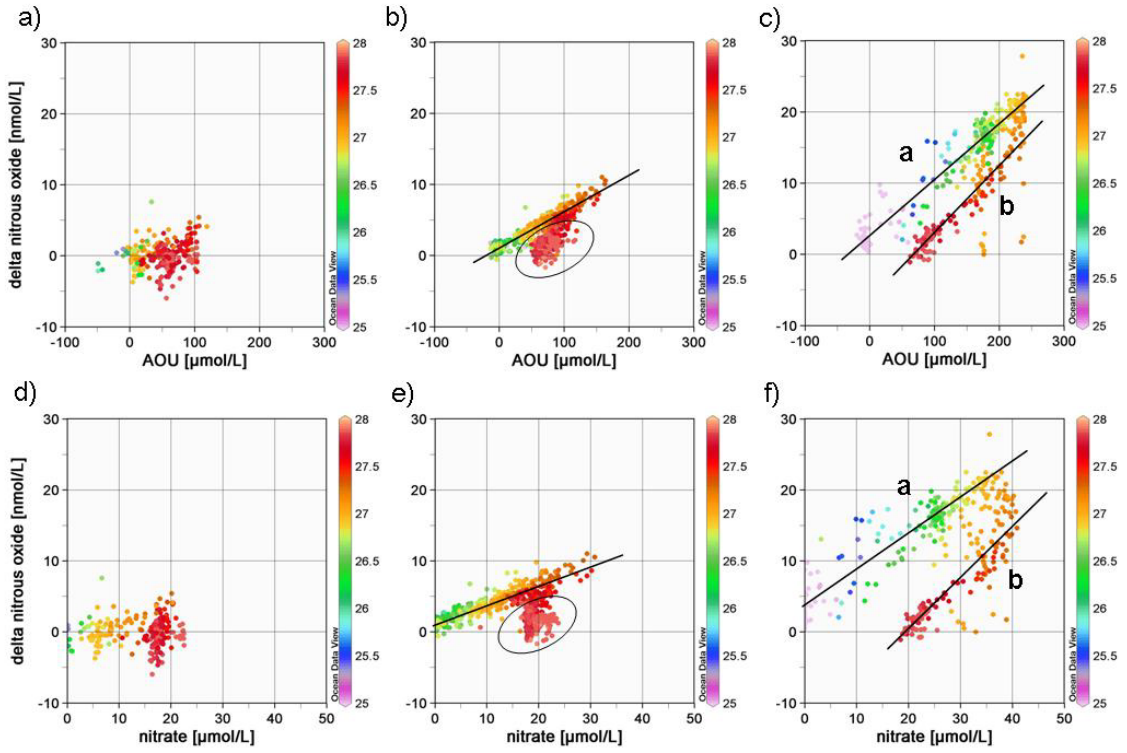

Fig. 7: ΔN_2O in comparison with AOU (7a-c) and nitrate (7d-f), sigma σ_θ is colour coded in kg m^{-3}.
7a and 7d) subpolar North Atlantic
7b and 7e) subtropical North Atlantic;
y (ΔN_2O) = 0.0473x (AOU) + 1.1409, R^2 = 0.86 for σ_θ < 27.7 (~ <1000m);
y (ΔN_2O) = 0.2497x (NO_3^-) + 1.1776, R^2 = 0.80 for σ_θ < 27.7 (~ <1000m);
circled data represent σ_θ > 27.7(~ >1000m)
7c and 7f) tropical North Atlantic;
line a: y (ΔN_2O) = 0.0785x (AOU) + 2.4381, R^2 = 0.87 for σ_θ < 27.1 (~ <500m);
line b: y (ΔN_2O) = 0.0942x (AOU) – 6.6675, R^2 = 0.81 for σ_θ > 27.1 (~ >500m);
line a: y (ΔN_2O) = -0.4848x (NO_3^-) + 3.1756, R^2 = 0.79 for σ < 27.1 (~ <500m);
line b: y (ΔN_2O) = 0.7379x (NO_3^-) – 14.665 R^2 = 0.79 for σ > 27.1 (~ >500m)

Since a multiple regression analysis turned out to be not applicable due to the co-linearity between the independent variables nitrate and AOU, we applied simple regression analysis for the isopycnal levels below the thermocline (see Tab. 2). Above the thermocline in the surface layer no correlations were found.

In the subpolar North Atlantic (Fig. 7a, d) ΔN_2O is low, with values near zero. There were no significant correlations found with AOU (Fig. 7a) or nitrate (Fig. 7d; see Tab. 2).

In the subtropical North Atlantic (Fig. 7b, e) ΔN_2O was also low with values ranging from 0 to 10 nmol L^{-1}. In contrast to the subpolar North Atlantic we found significant correlations between ΔN_2O, AOU (Fig. 7b) and nitrate (Fig. 7e; see Tab. 2), especially at depths down to the N_2O maxima (ca. 1000 m; $\sigma_\theta < 27.7$; see regression lines in Fig. 7b and 7e). Below 1000 m (σ_θ 27.7) ΔN_2O did not correlate with AOU or NO_3^- (circled data in Fig. 7b and 7e).

In the tropical North Atlantic (Fig. 7c, f) correlations between ΔN_2O, AOU (Fig. 7c) and nitrate (Fig. 7f) were more pronounced. We observed different ΔN_2O/AOU ratios at depths down to ΔN_2O maxima and below the ΔN_2O maxima down to the bottom. From the surface layer down to 500 m ($\sigma_\theta < 27.1$; see regression line a) the slope of the regression line (ΔN_2O/AOU) was approximately 20 % lower than at depths below 500 m ($\sigma_\theta > 27.1$; see regression line b), what implies that the yield of N_2O at equal AOU is lower at shallower depths.

Table 2: Regression analyses between ΔN_2O and AOU, and ΔN_2O and NO_3^- at different isopycnal levels. Bold numbers mean relationships with significance levels of $p < 0.001$ and $R^2 > 0.5$. The coefficients a and b mean slope and intercept.

			ΔN_2O / AOU			**ΔN_2O / NO_3^-**		
region	**sigma**	**n**	**a**	**b**	**R^2**	**a**	**b**	**R^2**
subpolar	thermocline - 26.0	-	-	-	-	-	-	-
	26.1 - 26.5	-	-	-	-	-	-	-
	26.6 - 27.0	13	0.045	-0.209	0.21	0.205	-0.882	0.09
	27.1 - 27.5	49	0.028	-0.452	0.23	0.159	-1.087	0.14
	27.6 - 27.9	179	0.022	-1.714	0.07	0.164	-3.240	0.03
subtropical	thermocline - 26.0	-	-	-	-	-	-	-
	26.1 - 26.5	45	**0.038**	**1.307**	**0.58**	**0.297**	**1.337**	**0.51**
	26.6 - 27.0	106	**0.065**	**0.397**	**0.75**	**0.390**	**0.186**	**0.68**
	27.1 - 27.5	121	**0.055**	**0.426**	**0.67**	**0.312**	**-0.052**	**0.60**
	27.6 - 27.9	355	0.069	-3.083	0.38	-0.126	4.579	0.02
tropical	thermocline - 26.0	34	**0.080**	**3.783**	**0.72**	**0.599**	**4.681**	**0.61**
	26.1 - 26.5	39	**0.099**	**-1.421**	**0.83**	**0.777**	**-3.212**	**0.81**
	26.6 - 27.0	98	0.107	-3.112	0.49	0.376	6.068	0.20
	27.1 - 27.5	63	0.114	-10.638	0.43	1.289	-35.05	0.42
	27.6 - 27.9	69	**0.075**	**-4.853**	**0.67**	**0.619**	**-11.83**	**0.66**

5. Discussion

Based on our results, we were able to assign measured N_2O concentrations to the water masses as shown in Figure 2a (Fig. 8). In the following we discuss distributions and possible origins of nitrous oxide at different depths with regard to these water masses.

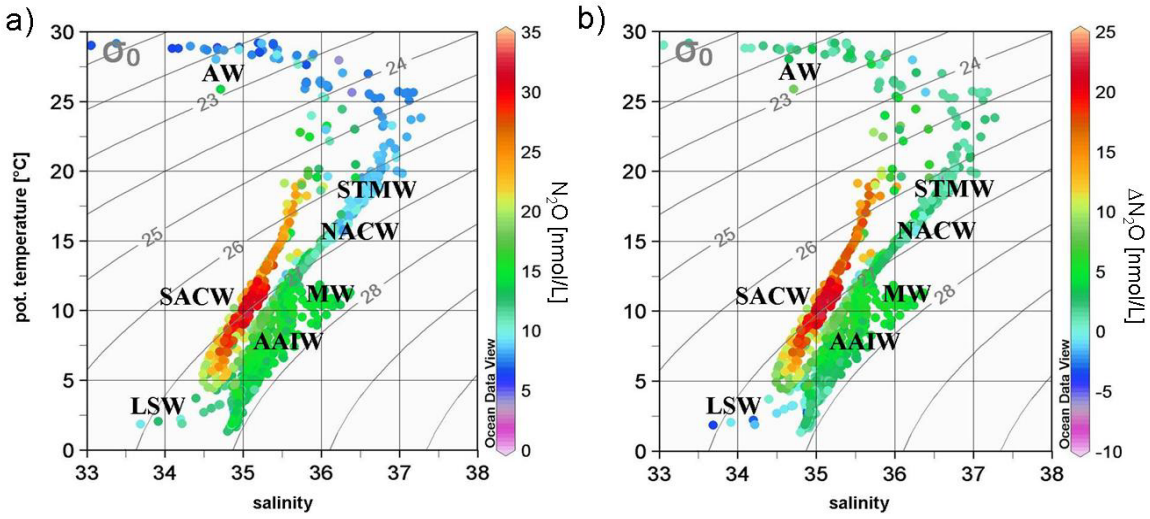

Fig. 8: N_2O concentration (a) and ΔN_2O (b) distributed in a T-S-diagram, the N_2O and ΔN_2O concentrations are colour coded in nmol L^{-1}. AW: Amazon Water; STMW: Subtropical Mode Water; MW: Mediterranean Water; SACW: South Atlantic Central Water; NACW: North Atlantic Central Water; AAIW: Antarctic Intermediate Water; LSW: Labrador Sea Water

5.1 N_2O in the surface layer of the North Atlantic

In the surface layer of the North Atlantic the distribution of N_2O was relatively uniform, with concentrations near equilibrium. This is in line with the assumption that denitrification and nitrification as sources of nitrous oxide in the surface layer seem to be negligible due to the high oxygen concentrations and light inhibition of nitrification [*Horrigan et al.*, 1981]. Thus, correlations between ΔN_2O, AOU and nitrate were nonexistent. Accordingly, we suggest that the N_2O distribution in the surface layer is most likely driven by solubility and mixing effects. This is also applicable for the enhanced N_2O concentrations found in the Labrador Current. The ΔN_2O concentrations, which are corrected for temperature, showed no enhanced values in this region. Thus, higher N_2O concentrations in the Labrador Current are likely caused by the solubility effect as well. In the warmer surface layer of the tropical North Atlantic ΔN_2O values were up to 4 nmol L^{-1}, indicating the tropical North Atlantic acts as a weak source for atmospheric N_2O [*Walter et al.*, 2004].

5.2 N_2O below the surface layer down to 2000 m

Variations of N_2O vertical profiles reflected effects of water mass ventilation and subsurface N_2O production history. In the subpolar North Atlantic we assume that the hydrographic setting (such as convection processes during deep water formation and vertical mixing) is responsible for the observed concentrations and distributions of N_2O and ΔN_2O. The most important feature in the subpolar North Atlantic is the formation of deep water in winter in the Labrador and Irminger Seas as part of the North Atlantic circulation in 500 to 2000 m [*Rhein*, 2000], which carries the atmospheric N_2O imprint to depth. Labrador Sea Water spreads rapidly east- and southwards [*Rhein*, 2000] and thus causes the uniform distribution of N_2O within the subpolar North Atlantic. Although the productivity of phytoplankton is relatively high in the subpolar North Atlantic, we assume low biological production of N_2O because of two factors: 1) high oxygen concentrations and 2) low temperatures. The yield of N_2O depends on oxygen concentration [*Goreau et al.*, 1980; *Poth and Focht*, 1985; *Richardson*, 2000; *Codispoti et al.*, 1992], whereas high oxygen concentrations weaken the production of N_2O. Furthermore the low temperatures of the North Atlantic might have been crucial as well. The temperature dependence of both nitrification rates and enzyme activities is controversially discussed [*Berounsky and Nixon*, 1990; *Vouve et al.*, 2000; *Barnard et al.*, 2005; *Herbert*, 1999; *Rheinheimer*, 1964; *Hansen et al.*, 1981; *Rysgaard et al.*, 1996], however growth rates and biological production of bacteria clearly depend on the prevailing temperatures [*Bock and Wagner*, 2001; *Hoppe et al.*, 2002]. Thus, N_2O production might not be limited directly by temperature but indirectly by the limited abundance of N_2O producing bacteria.

In the subtropical North Atlantic concentrations of N_2O and ΔN_2O were distinctly higher compared to the subpolar North Atlantic. Profiles differed clearly between the western and eastern basin. N_2O profiles in the western basin showed clearly expressed N_2O maxima between 600 to 1000 m. This pattern was not observable east of the Midatlantic Ridge where N_2O and ΔN_2O concentrations were lower than in the western basin, and no peak maxima were observed. Hydrographic processes likely explain the shape of profiles, especially the advection of Labrador Sea Water (LSW) into the eastern basin. LSW with low N_2O concentrations is transported either along the eastern continental slope of America or across the Charlie-Gibbs-Fracture-Zone [*Bower et al.*, 2002]. It flows into the eastern subtropical basin at 500-2000 m [*Rhein*, 2000; *Alvarez et al.*, 2004], and

spreads north- and southwards [*Bower et al.*, 2002; *Rhein*, 2000]. In the western basin N_2O concentrations and profiles are in agreement with profiles published by [*Yoshinari*, 1976], who also found maximum values in water masses with lower oxygen concentrations. These were identified as Antarctic Intermediate Water (AAIW), which flows northwards. We assume the AAIW transports N_2O from the south to the subpolar North Atlantic. At depths shallower than 1000 m ($\sigma_\theta < 27.7$) ΔN_2O was significantly correlated with oxygen utilization and nitrate concentrations (Fig. 7b, 7e; Tab. 2), indicating nitrification has contributed to measured N_2O concentrations.

In the tropical North Atlantic the N_2O profiles and the observed trend along the West-East transect are in overall agreement with recently published data from a transect along 7.5 °N [*Oudot et al.*, 2002] and a previous study in the Guinea Dome area [*Oudot et al.*, 1990]. Although the overall pattern is the same, we observed generally lower N_2O concentrations than *Oudot et al.* [2002]. This might be a result of a calibration disagreement, supported by measured atmospheric N_2O values of 316 ppb in 1993. For example, we found a peak N_2O concentration of up to 37.3 nmol L^{-1} in South Atlantic Central Water (SACW) of the eastern basin, whereas *Oudot et al.* [2002] reported values of up to 60 nmol kg^{-1}. *Oudot et al.* [2002] assumed enhanced biological activity and remineralization of organic matter in upwelling ecosystems to be responsible for these higher values in the east. However, upwelling in this area is a temporary event [*Voituriez et al.*, 1982; *Siedler et al.*, 1992], and during our cruise no upwelling was observed.

Despite the fact that upwelling might have a long-term large-scale effect, we suppose additional reasons for the higher N_2O concentrations in the eastern basin. The productivity in the eastern basin is fueled not only by coastal upwelling [*Signorini et al.*, 1999] but also by dust deposition off the West African coast [*Mills et al.*, 2004]. Moreover, nutrient input by major tropical rivers such as the Senegal, Gambia and Niger [*Perry et al.*, 1996] contribute to enhanced production off the West African coast, indicated by enhanced chlorophyll a concentrations (for the 2002 seasonal cycle of chlorophyll a see monthly data set of Sea-viewing Wide Field-of-view Sensor (SeaWiFS): http://earthobservatory.nasa.gov/Observatory/Datasets/chlor.seawifs.html.

Enhanced productivity leads to a high export production [*Antia et al.*, 2001]. Subsequently lowered O_2 concentrations in the eastern intermediate layers due to the demineralization of organic matter support the production of N_2O. Therefore, enhanced N_2O

concentrations in the eastern basin at this time of year (Oct./Nov. 2002) might be a residual signal of past high production episode [*Signorini et al.*, 1999].

Upwelling events, indicated by lower sea surface temperatures, were only found at the equator. Surface N_2O concentration and sea surface temperature were positively correlated [*Walter et al.*, 2004], and the comparably shallow N_2O maxima along the equator were caused by upwelling.

Due to the occurrence of linear relationships between ΔN_2O and AOU and between N_2O and nitrate we conclude that nitrification might be the major pathway of N_2O formation in the tropical Atlantic Ocean [*Yoshida et al.*, 1989]. Like N_2O, ΔN_2O showed an increasing trend from West to East indicating that nitrification is more pronounced in the eastern than the western basin of the tropical Atlantic.

5.3 N_2O in deep waters > 2000 m (σ_θ > 27.8)

Because the deep ocean contains high nitrate concentrations, nitrification was assumed to be responsible for N_2O production [*Zehr and Ward*, 2002; *Bange and Andreae*, 1999]. Due to the low ΔN_2O in deep waters and insufficient correlations with nitrate and AOU, we assume N_2O at these depths probably originates from deep water formation and mixing processes of southern and northern hemisphere water masses. N_2O profiles from a cruise into the Antarctic circumpolar current (2 °E / 49.5 °) [Walter et al., in press] and BLAST II data east of Patagonia reveal distinctly higher N_2O concentrations in the deep waters of the southern hemisphere, with values of approximately 17 nmol L^{-1}. Northwards transport within Antarctic Bottom Water could lead to enhanced N_2O concentrations in the deep water of the North Atlantic by mixing and diffusion process.

6. Conclusions

N_2O concentrations in the North Atlantic showed characteristic variations in the vertical and horizontal distributions. In general, distribution of N_2O can be explained by a combination of biological and hydrographic reasons. The main conclusions of the present study are

- Production of N_2O by nitrification occurs mainly in the tropical North Atlantic, especially in the eastern basin. Maximum values were found in the Antarctic Intermediate Water (AAIW) in the western basin, and in the South Atlantic Central Water (SACW) in the eastern basin.
- Vertical N_2O distribution and shape of profiles in the subtropical North Atlantic originate from production by nitrification and advection of AAIW from the south into the western subtropical North Atlantic, respectively advection of LSW from the north in the eastern subtropical North Atlantic.
- In the subpolar North Atlantic mainly mixing processes may control the distribution of N_2O, particularly the deep water formation in the Labrador Sea. Production seems to be negligible.
- Tropical and subtropical regions showed supersaturation throughout the water column, thus the tropical and subtropical North Atlantic act as a source of atmospheric N_2O.
- Outflow water of the Amazon or the Mediterranean Sea does not affect the N_2O concentration.

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Chapter 3

Nitrous oxide in the surface layer of the tropical North Atlantic Ocean along a West to East transect

Sylvia Walter, Hermann W. Bange, Douglas W. R. Wallace

Abstract

Nitrous oxide (N_2O) was measured during the first German SOLAS (Surface Ocean – Lower Atmosphere Study) cruise in the tropical North Atlantic Ocean onboard R/V *Meteor* during October/November 2002. About 900 atmospheric and dissolved N_2O measurements were performed with a semi-continuous GC-ECD system equipped with a sea-water-gas equilibrator. Surface waters along the main transect at 10°N showed no distinct longitudinal gradient. Instead, N_2O saturations were highly variable ranging from 97% to 118% (in the Guinea Dome Area, 11°N, 24°W). When approaching the continental shelf of West Africa, N_2O surface saturations went up to 113%. N_2O saturations in the region of the equatorial upwelling (at 0–1.5 °N, 23.5–26 ° W) were correlated with decreasing sea surface temperatures and showed saturations up to 109%. The overall mean N_2O saturation was 104 ± 4 % indicating that the tropical North Atlantic Ocean is a net source of atmospheric N_2O.

Introduction

Nitrous oxide (N_2O) is an important atmospheric trace gas because it influences, directly and indirectly, the Earth's climate to a significant degree: In the troposphere, it acts as a greenhouse gas with a relatively long atmospheric lifetime [*IPCC*, 2001] whereas in the stratosphere it is the major source for nitric oxide radicals, which are involved in one of the main ozone reaction cycles [*WMO*, 2003]. Published source estimates indicate that the world's oceans play a major role in the global budget of atmospheric nitrous oxide [*IPCC*, 2001]. Generally, oligotrophic areas seem to be near equilibrium with the atmosphere, whereas coastal and equatorial upwelling areas show enhanced N_2O concentrations [*Nevison et al.*, 1995; *Suntharalingam and Sarmiento*, 2000]. Here we present about 900 measurements of dissolved and atmospheric N_2O during the first German SOLAS (Surface Ocean – Lower Atmosphere Study) cruise. It is the first high-resolution data set of N_2O in the tropical North Atlantic Ocean along a West to East transect and it is complementary to previous N_2O measurements of *Oudot et al.* [1990; 2002] and *Weiss et al.* [1992].

The cruise took place onboard R/V *Meteor* (expedition no. M55) from Willemstad (Curaçao, Netherl. Antilles) to Douala (Cameroon) from 12 October to 17 November 2002. The cruise track consisted of two main transects: (i) The West to East transect along 10-12°N covering the oligotrophic tropical North Atlantic Ocean and the continental shelf area of the West African coast off Guinea Bissau and (ii) a shorter West to East transect along the equatorial upwelling (Fig. 1).

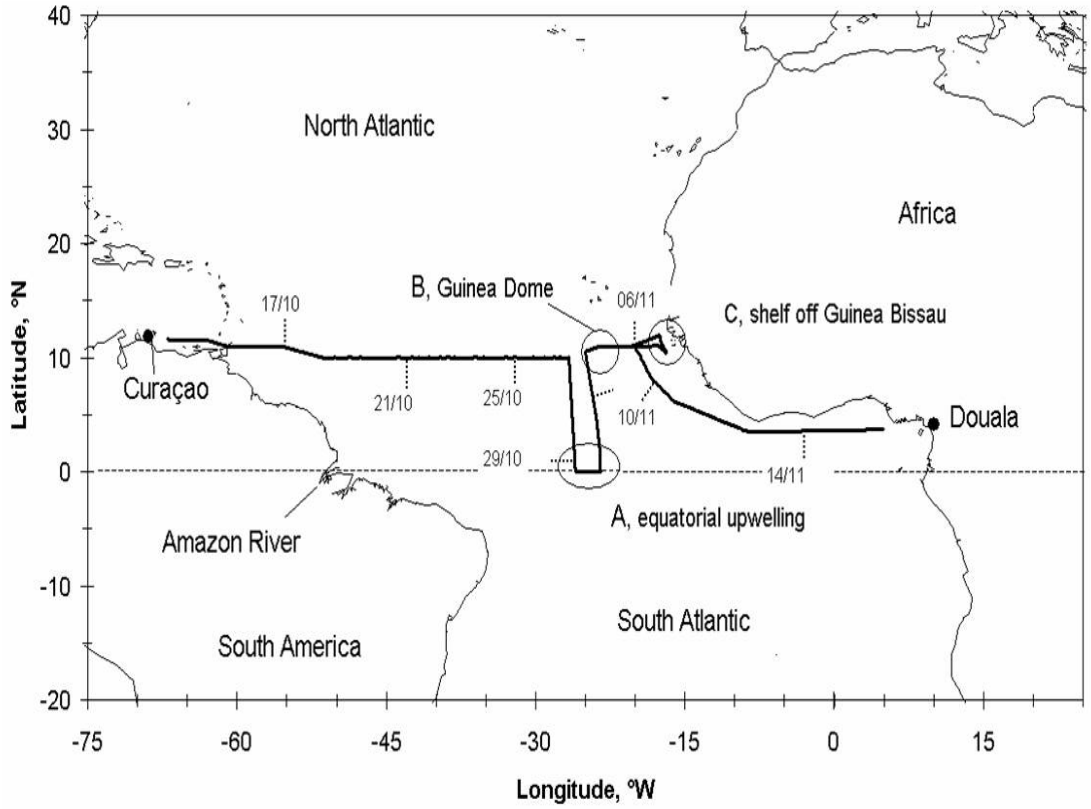

Fig. 1: Cruise track of M55 in October–November 2002. N_2O measurements were started 17 October and were finished 14 November. Areas of special interest discussed in the text are marked.

1. Method

N_2O was determined with a gas chromatograph equipped with an electron capture detector. Further details of the analysis system are described in *Bange et al.* [1996]. A series of measurements of atmospheric N_2O and N_2O in seawater-equilibrated air followed by two standards was repeated every 50 min. Mixtures of N_2O in synthetic air were used to obtain two-point calibration curves. The mixtures used contained 311.7 ± 0.1 and 346.5 ± 0.2 ppb N_2O, respectively. These are gravimetrically prepared gas mixtures (Deuste Steininger GmbH, Mühlhausen, Germany) and have been calibrated against the NOAA (National Oceanic and Atmospheric Administration, Boulder, Co.) standard scale in the laboratories of the Air Chemistry Division of Max Planck Institute for Chemistry Mainz, Germany. The precision, calculated as the ratio of the standard deviation of the atmospheric measurements and the mean atmospheric mixing ratio, was 0.8%.

Seawater was pumped continuously from a depth of 4 m into a shower-type equilibrator developed by R. F. Weiss (Scripps Institution of Oceanography, La Jolla, Ca.). N_2O con-

centrations (C, in nmol L^{-1}) were calculated by applying the solubility equation of *Weiss and Price* [1980]:

$$C = \beta(T, S)\, x' \, P,$$

where x' is the measured N_2O dry mole fraction, P is the atmospheric pressure, and β is the solubility coefficient, which is a function of the water temperature (T) and salinity (S). Time series of surface seawater temperature (SST), salinity, wind speed, and atmospheric pressure were obtained from the ship's records. Differences between the seawater temperature at the seawater intake and the continuously recorded water temperature in the equilibrator were corrected:

$$C_w = C\, \beta(T_{eq}) / \beta(SST)$$

with $\beta(SST)$ and $\beta(T_{eq})$ representing the N_2O solubility at seawater temperature and water temperature inside the equilibrator at the time of the measurement, respectively. N_2O saturations (Sat) in % (i.e., 100% = equilibrium) were calculated as follows:

$$Sat = 100\, C_w / C_a$$

where C_a is the equilibrium concentration of dissolved N_2O based on the actual measurement of ambient air (see above). The mean relative errors of the N_2O concentrations and saturations were calculated to be 1.2 % and 1.6 %, respectively (details of the error propagation computation are given in *Bange et al.* [2001]).

2. Results and Discussion

The mean atmospheric N_2O dry mole fraction was 318 ± 3 ppb. Due to the seasonal northward shift of the Intertropical Convergence Zone to about 10°N, the origin of the air masses sampled during the cruise were from both the northern and the southern hemisphere. 4-days air mass back trajectories (provided by the German Weather Service, Offenbach, Germany) indicated that air masses sampled at latitudes south of 7°N originated from the southern hemisphere. Based on this classification we computed mean N_2O values for northern and southern hemisphere air masses of 319 ± 3 ppb and 317 ± 2 ppb, respectively. The observed atmospheric values are in agreement with N_2O measurements at the baseline monitoring stations Ragged Point, Barbados and Cape Grim, Tasmania. Monthly mean values were 317 ppb (Cape Grim) and 318 ppb (Ragged Point) for October/November 2002. These values were taken from the Advanced Global Atmospheric Gases Experiment (AGAGE) data set (updated version from November 2003) [*Prinn et al.*, 2000]. AGAGE data are available from the anonymous ftp site: cdiac.esd.ornl.edu (subdirectory /pub/ale_gage_Agage/Agage/gc-md/monthly) at the Carbon Dioxide Information Analysis Center in Oak Ridge, Tennessee.

N_2O saturations along the main cruise track ranged from 97% to 118% and the SST was generally between 27 and 30 °C (Fig. 2). Since the main cruise track was located between the eastward flowing North Equatorial Countercurrent (NECC) and the westward flowing North Equatorial Current (NEC) [*Stramma and Schott*, 1999], we crossed several times meandering waters of different origins causing a high variability of the N_2O saturation: Low N_2O saturations of about 100% observed around 24 Oct., 27-28 Oct., and 2 Nov. were generally associated with decreases in salinity (Fig. 2).

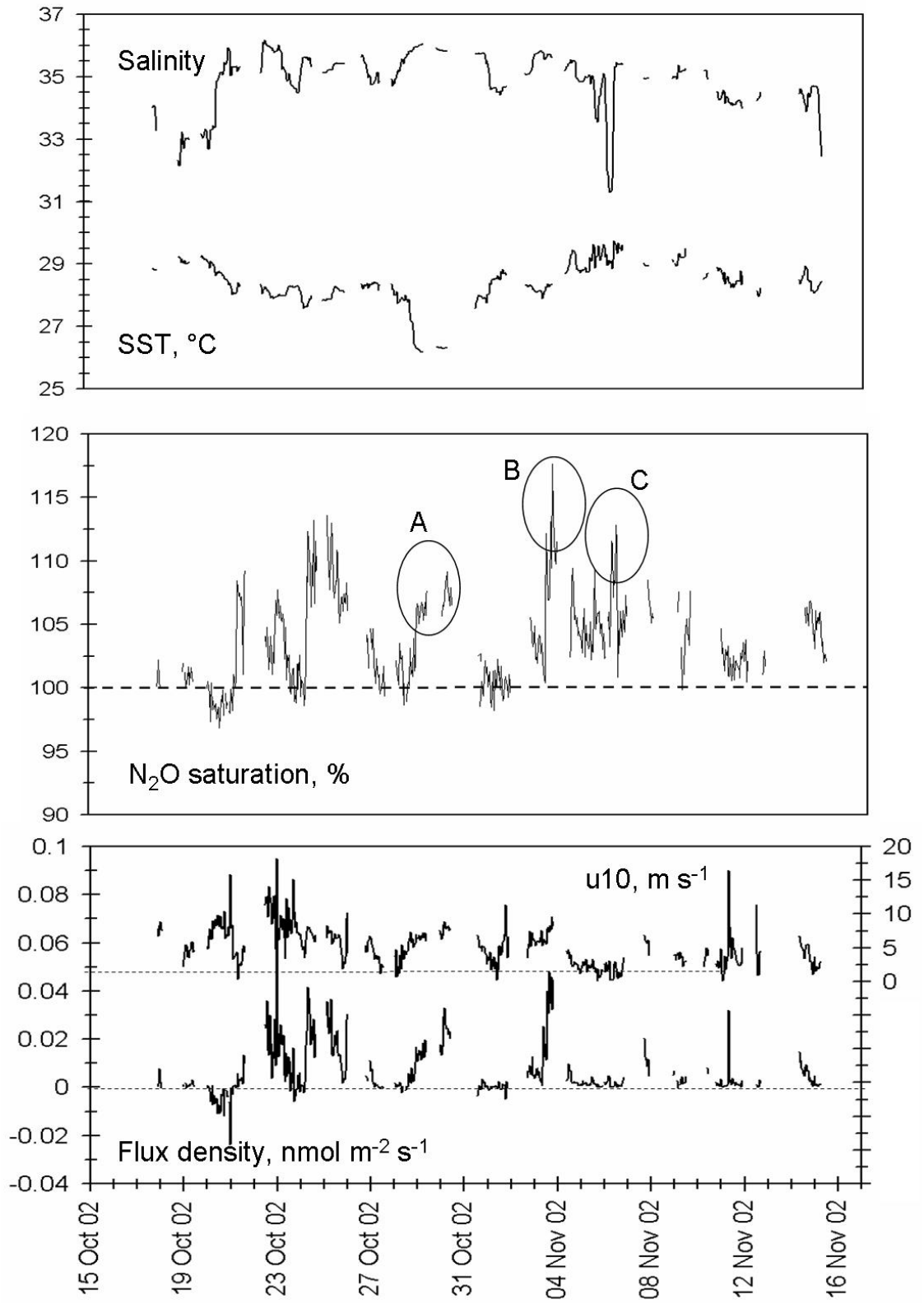

Fig. 2: Salinity, seasurface temperature (SST), N_2O saturation, wind speed in 10 m height (u10), and N_2O flux density during M55. Areas of special interest discussed in the text are marked (see Fig. 1): A, equatorial upwelling; B, Guinea Dome; C, shelf of West Africa (water depths <200m).

This results from the retroflection of the North Brazil Current, which advects Amazon plume waters (with low N_2O, see below) eastward into the NECC [*Fratantoni and Glickson*, 2002]. Freshwater influences were observed twice: First, at around 50°E (19 Oct., Fig. 2) when we crossed the northern boundary of the Amazon River plume (minimum salinity 32.14) and second, on the continental shelf off West Africa where we measured a drop in salinity down to 31.30 (5-6 Nov., Fig. 2). N_2O saturations were not enhanced in the Amazon River plume, whereas an increase in N_2O saturations up to 113% was observed on the West African shelf. The low N_2O saturations in the Amazon River plume were attributed to the fact that N_2O-rich waters from the Amazon River are N_2O-depleted because of outgasing to the atmosphere and mixing with near-equilibrium oceanic waters while distributed to the North [*Oudot et al.*, 2002]. The high N_2O saturations on the continental African shelf might result from N_2O-rich riverine waters or groundwater seepage, but not from coastal upwelling as indicated by the uniform SSTs. N_2O saturations up to 118% were observed in the area of the Guinea Dome at 11°N, 24°W (3-4 Nov., Fig. 2) which is well-known for pronounced Ekman upwelling [*Siedler et al.*, 1992; *Signorini et al.*, 1999]. In the equatorial region (0-1.5°N, 28-30 Oct., Fig. 2) SSTs dropped well below 27°C and were associated with enhanced N_2O saturations (up to 109%). We found a good correlation between N_2O concentrations and SST in the equatorial upwelling region (Fig. 3) indicating that the enhanced surface N_2O saturations were resulting from upwelling of N_2O-enriched subsurface waters.

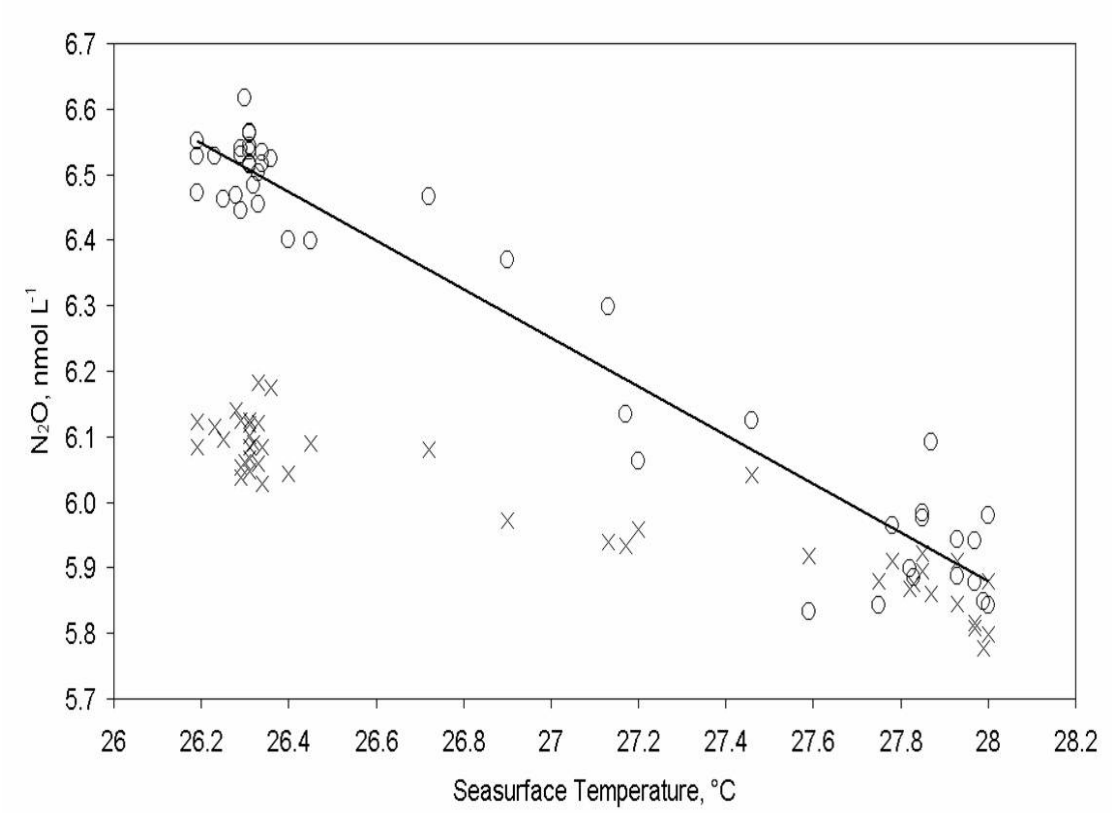

Fig. 3: Correlation ($r^2 = 0.94$, n = 46) of SST and N_2O concentration in the equatorial upwelling area (0–2°N, 23.5–26°W). Open circles stand for in-situ measurements and crosses stand for the corresponding equilibrium concentrations calculated as a function of the actual SST, salinity, atmospheric dry mole fraction, and ambient pressure.

In order to account for the NECC/NEC system and the observed N_2O features we defined two latitudinal aligned open ocean regions and a shelf region: 1) the tropical North Atlantic ranging from 1.5-12°N with SST >27°C, 2) the equatorial upwelling from 0-1.5°N with SST <27°C, and 3) the shelf area off the West African coast (water depth <200m). An overview of the regional mean concentrations and saturations is given in Table 1.

Table 1: Mean N_2O concentrations, saturations, and flux densities during M55. Values are given as mean ± 1sd. Number of measurements is given in parenthesis.

	Overall mean (n = 451)	0 – 1.5 °N (n = 27)	1.5 – 12°N (n = 416)	Shelf (n = 8)
Concentration, nmol L^{-1}	6.00 ± 0.24	6.49 ± 0.07	5.27 ± 0.20	6.31 ± 0.11
Saturation, %	104 ± 4	107 ± 1	103 ± 3	110 ± 2
Flux density, nmol m^{-2} s^{-1}	0.007 ± 0.011	0.018 ± 0.006	0.006 ± 0.011	0.002 ± 0.002

The mean N_2O concentrations and saturations of the shelf and equatorial regions are significantly enhanced compared to the 1.5-12°N region. The high variability calculated for the tropical North Atlantic region is biased by the complex hydrography, which is influenced by the Amazon plume, the NECC/NEC system and the Guinea Dome upwelling with highly variable N_2O concentrations. However, a more detailed regional analysis is hampered by the limited data set.

Our data from the tropical North Atlantic are in agreement with previously published data. Recently, *Oudot et al.* [2002] reported a mean N_2O saturation of 108 ± 3 %, mainly measured along two transects at 7.5°N and 4.5°S during January–March 1993. They also observed a trend towards enhanced values when approaching the West African coast (up to 118%). In a previous study in the Guinea Dome area during June-August 1986, *Oudot et al.* [1990] observed mean N_2O saturations in the range from 126 ± 5 to 132 ± 6 % which are considerably higher than our results (Table 1). In the period from 1979 to 1989, *Weiss et al.* [1992] took part in several measurement campaigns with cruise tracks across the tropical North Atlantic Ocean. Their N_2O measurements are in good agreement with the results presented here. For example, the mean N_2O saturation during the first part of the TTO/TAS leg 3 in February 1983, which covered two transects along 9.5°N (from 20.25 to 28 °W) and 28°W (from 9.5 to the equator), was about 105 %. Enhanced values during TTO/TAS leg 3 were observed on the coast off Guinea-Bissau (up to 179%) and in the equatorial upwelling (up to 111%). In contrast to our measurements, the high N_2O values observed off Guinea Bissau were caused by coastal upwelling (SST <27°) [*Weiss et al.*, 1992]. Summarizing the results from various N_2O measurements in the open tropical North Atlantic, we found only slight differences (with the exception of the data from the Guinea Dome area by *Oudot et al.* [1990]). Significant differences as found for the Guinea Dome might be caused by seasonal variability of the circulation patterns [*Stramma and Schott*, 1999] in connection with different spatial data coverage. Since coastal upwelling was absent during our cruise, N_2O saturations on the shelf off West Africa were comparably low.

2.1 N_2O air-sea exchange

The air–sea exchange flux density (F) was parameterized as

$$F = k_w(u)\ (C_w - C_a),$$

where k_w (in m s^{-1}) is the gas transfer coefficient as a function of wind speed (u in 10 m height), C_w is the measured N_2O seawater concentration, and C_a is the equilibrium N_2O concentration in seawater based on the measured atmospheric value (for calculation of C_w and C_a see Methods section). To calculate k_w, we used the combined linear and quadratic k_w–u relationship (N00) from *Nightingale et al.* [2000]:

$$k_w = 9.25\ 10^{-5}\ u + 6.17\ 10^{-7}\ u^2.$$

The N00 relationship shows a dependence on wind speeds intermediate between the commonly used relationships of *Liss and Merlivat* [1986] and *Wanninkhof* [1992]. The measured wind speeds were normalized to 10 m height by using the relationship of *Garratt* [1977]. k_w was adjusted by multiplying with $(Sc/600)^{-0.5}$, where Sc is the *Schmidt* number for N_2O. Sc was calculated using empirical equations for the kinematic viscosity of seawater [*Siedler and Peters*, 1986] and the diffusion coefficient of N_2O in water. N_2O diffusion coefficients (D_{N2O} in m^2 s^{-1}) were calculated with the equation derived from a compilation of actual measurements [*Rhee*, 2000]:

$$D_{N2O} = 3.16\ x\ 10^{-6}\ exp\ (-18370 / RT),$$

where T is the water temperature in K and R is the universal gas constant. The commonly used equation for D_{N2O} by *Broecker and Peng* [1974] was replaced since *Rhee*'s [2000] equation provides a more reasonable fit with a considerably reduced uncertainty of less than 10% [*Rhee*, 2000]. Flux densities calculated with the above equation are lower by about 10% when compared to computations with *Broecker and Peng*'s [1974] equation [*Bange et al.*, 2001]. We did not apply a correction of D_{N2O} for seawater since the effect of seawater on the diffusion of dissolved gases is not uniform [*King et al.*, 1995] and, to our knowledge, no measurements of the N_2O diffusion in seawater have been published.

The regional mean flux densities clearly reflect the interplay of saturation and wind speeds (Fig. 2, Tab. 1). In the equatorial region enhanced N_2O saturations and compara-

bly high wind speeds result in high flux densities, whereas over the shelf enhanced N_2O saturations were associated with very low wind speed resulting in low flux densities (Fig. 2). The mean flux density of the tropical North Atlantic region is biased by the high variability of both N_2O saturations and wind speeds. The overall mean N_2O flux density was 0.007 ± 0.011 nmol m^{-2} s^{-1} which is at the lower end of previously published flux densities: *Oudot et al.* [1990; 2002] computed overall mean flux densities of 0.013–0.021 nmol m^{-2} s^{-1} and 0.026 ± 0.032 nmol m^{-2} s^{-1} for the tropical North and South Atlantic and the Guinea Dome area, respectively. The obvious discrepancy might be caused by different spatial data coverage, seasonal variability of the N_2O concentrations and wind speeds, and the use of different approaches for the transfer coefficient k_w.

3. Summary

N_2O saturations in the tropical North Atlantic Ocean during October–November 2002 were highly variable and range from 97 to 118 %. The mean overall saturation was 104 ± 4 %. Enhanced saturations were observed in the Guinea Dome area (up to 118%), in the equatorial upwelling (up to 109%), and the shallow continental shelf area off the West African Coast (up to 113%). Our results are in agreement with previously published data sets. We found a good correlation of seawater temperature with N_2O concentrations in the equatorial upwelling area. We conclude that the tropical North Atlantic Ocean is a net source of N_2O to the atmosphere with a pronounced regional variability.

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Chapter 4

Nitrous oxide measurements during EIFEX, the European Iron Fertilisation Experiment in the subpolar South Atlantic Ocean

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Abstract

We measured the vertical water column distribution of nitrous oxide (N_2O) during the European Iron Fertilization Experiment (EIFEX) in the subpolar South Atlantic Ocean during February/March 2004 (R/V *Polarstern* cruise ANT XXI/3). Despite a huge build-up and sedimentation of a phytoplankton bloom, a comparison of the N_2O concentrations within the fertilized patch with concentrations measured outside the fertilized patch revealed no N_2O accumulation within 33 days. This is in contrast to a previous study in the Southern Ocean, where enhanced N_2O accumulation occurred in the pycnocline. Thus, we conclude that Fe fertilization does not necessarily trigger additional N_2O formation and we caution that a predicted radiative offset due to a Fe-induced additional release of oceanic N_2O might be overestimated. Rapid sedimentation events during EIFEX might have hindered the build-up of N_2O and suggest, that not only the production of phytoplankton biomass but also its pathway in the water column needs to be considered if N_2O radiative offset is modeled.

1. Introduction

Inspired by the iron (Fe) limitation hypothesis [*Martin et al.*, 1991], several Fe fertilization experiments have been performed in high nutrient-low chlorophyll (HNLC) regions such as the Southern Ocean, and the subarctic and equatorial Pacific Ocean [see e.g., *Boyd*, 2004; *Boyd*, 2002]. *Fuhrman and Capone* [1991] pointed out that stimulating ocean productivity by Fe addition might result in an enhanced formation of nitrous oxide (N_2O). This point is especially important in view of the fact that N_2O is an atmospheric trace gas with a high global warming potential [*Jain et al.*, 2001]. Thus, enhanced N_2O formation by Fe addition might counteract the climatic benefits of a drawdown of atmospheric carbon dioxide (CO_2).

Fuhrman and Capone [1991] argued that enhanced productivity will lead to an enhanced nitrogen export from the euphotic zone, which in turn would result in additional N_2O formation via enhanced nitrification ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$). N_2O formed via nitrification is thought to be dominating in the oxic part of the world's oceans [see e.g., *Nevison et al.*, 2003]. The idea of a link between Fe fertilization and enhanced N_2O formation was supported by the study of *Law and Ling* [2001] who found a small but significant N_2O accumulation in the pycnocline during the Southern Ocean Iron Enrichment Experiment (SOIREE) in the Australasian sector of the Southern Ocean (61°S, 140°E) in February 1999. Recently, *Jin and Gruber* [2003] predicted the long-term effect of Fe fertilization on oceanic N_2O emissions on a global scale with a coupled physical-biogeochemical model. Based on their model results they concluded that Fe fertilization-induced N_2O emissions could offset the radiative benefits of the CO_2 drawdown [*Jin and Gruber*, 2003].

Here we present our measurements of N_2O during the European Iron Fertilization Experiment (EIFEX; R/V *Polarstern* cruise ANT XXI/3) in the subpolar South Atlantic Ocean from 9 February to 21 March 2004 [*Smetacek and cruise participants*, 2005].

1.1 The EIFEX setting

A mesoscale cyclonic eddy, embedded in a meander of the Antarctic Polar Front, was identified as suitable for the EIFEX study [*Strass et al.*, 2005]. The eddy was centered at 49.4°S 2.25°E and extended over an area of 60x100 km. First fertilization was performed on 12-13 February by releasing 6000 kg iron sulfate ($FeSO_4$) into the mixed layer over

an area of 150 km^2. Since iron concentrations had been decreasing [*P. Croot*, personal communication], fertilization was repeated on 26-27 February by releasing 7000 kg $FeSO_4$ over an area of 400 km^2. All sampled stations were located inside the eddy; the stations within fertilized waters will be called in-stations and those from unfertilized waters out-stations (Table 1). Inside and outside the fertilized patch was determined by photosynthetic activity (Fv/Fm) performed by Fast-Repetition-Rate-Fluorescence (FastTracka, Chelsea, UK) [*Röttgers et al.*, 2005]. Fv/Fm is known to be a very sensitive parameter, which increases immediately after iron fertilization.

The hydrographic settings of the sampling stations were not uniform: The in-stations' hydrographic properties did not show any variability. However, the out-station 514 showed, in comparison with the in-stations, enhanced potential water temperatures in the density (σ_t) range from 27.25 to 27.7 kg m^{-3} (corresponding to a approximate depth range from 200 to 400 m). The hydrographic properties of the out-stations 546 and 587 were almost identical to the in-stations. This implies that station 514 is not a representative out-station and was therefore excluded from the comparison (see also discussion of N_2O data below).

2. Methods

Triplicate water samples from various depths were taken with from a 24 x 12 L-bottle rosette, equipped with a CTD-sensor. The analytical method applied is a modification of the method described by *Bange et al.* [2001]: Bubble free samples were taken immediately following oxygen (O_2) sampling in 24 mL glass vials, sealed directly with butyl rubber stoppers and crimped with aluminium caps. To prevent microbial activity, samples were poisoned with 500 µL of a saturated aqueous mercury chloride ($HgCl_2$) solution. The samples were stored in the dark at 4 °C until analysis in our home laboratory from June to August 2004. In a time series experiment we found that N_2O concentrations in samples treated as described above did not change significantly over 10 months [*Walter*, 2005].

N_2O water concentrations (C_w) were calculated as follows:

$$C_w \left[\text{nmol L}^{-1}\right] = \left(\beta \; x' \; P \; V_{wp} + \frac{x' \; P}{R \; T} V_{hs} \right) / \; V_{wp}$$

where β stands for the Bunsen solubility in nmol L^{-1} atm^{-1} [*Weiss and Price*, 1980], x' is the dry gas mole fraction of N_2O in the headspace in ppb, P is the atmospheric pressure in atm (set to1 atm), V_{wp} and V_{hs} stand for the volumes of the water (14 mL) and headspace phases (10 mL), respectively. R is the gas constant (8.2054 10^{-2} L atm mol^{-1} K^{-1}) and T is the temperature during equilibration.

For calibration we used standard gas mixtures with 311.8 ± 0.2 ppb and 346.5 ± 0.2 ppb N_2O in synthetic air (DEUSTE Steininger GmbH, Mühlhausen, Germany). The standard mixtures have been calibrated against the NOAA (National Oceanic and Atmospheric Administration, Boulder, Co.) standard scale in the laboratories of the Air Chemistry Division of the Max Planck Institute for Chemistry, Mainz, Germany). The standard deviation of the N_2O concentration (C_w) was approximated with (C_{wmax} – C_{wmin}) / 1.91, where C_{wmin} and C_{wmax} stand for the minimal and maximal N_2O concentrations of the triplicate samples, respectively. The factor 1.91 is derived from the statistical method by *David* [1951]. The overall mean analytical error was ± 2.7% (± 0.5 nmol L^{-1}).

N_2O saturations (*sat*) in % (i.e., 100% = equilibrium) were calculated as $sat = 100 \; C_w / C_a$, where C_a is the equilibrium concentration of dissolved N_2O based on the N_2O atmospheric dry mole fraction, water temperature, and salinity [*Weiss and Price*, 1980]. For calculating C_a in the mixed layer an ambient air mole fraction of 317.8 ppb was applied, which is the average of the monthly mean N_2O dry mole fractions measured at the AGAGE (Advanced Global Atmospheric Gases Experiment, see *Prinn et al.* [2000]) baseline monitoring station Cape Grim (Tasmania) during February and March 2004. AGAGE data are available from the anonymous ftp site: cdiac.esd.ornl.edu (subdirectory /pub/ale_gage_agage/agage/gc-md/monthly) at the Carbon Dioxide Information Analysis Center in Oak Ridge, Tennessee.

Dissolved O_2, nitrate, and CTD data were provided by the participating working groups. Further details can be found in the cruise report by *Smetacek and cruise participants* [2005].

3. Results and Discussion

An overview of the N_2O measurements during EIFEX is given in Table 1 and in Figure 1. Mixed layer N_2O saturations were comparable to surface saturations (~ 103%) from the same region measured during the Ajax cruise leg 2 in Jan-Feb 1984 [*Weiss et al.*, 1992]. Moreover, the overall mean N_2O deep water (> 2000m) concentration of 17.5 ± 0.2 nmol L^{-1} is in good agreement with the N_2O deep water-water age relationship by *Bange and Andreae* [1999]. Both, the observed surface saturation and deep-water concentration support the view that the N_2O samples were not affected by the time lag between sampling and measurements.

In agreement with the results from SOIREE [*Law and Ling*, 2001], we did not observe a difference in N_2O mixed layer saturations between in-stations and out-stations (Table 1), which implies that N_2O emissions were not significantly different either.

Table 1: N_2O measurements during EIFEX. Class. stands for classification and indicates whether a profile was inside or outside of the fertilized patch. ML stands for mixed layer; here defined as the depth where the temperature differs from the surface temperature by more than 0.5 °C. Conc. and sat. stand for concentration and saturation, respectively.

Station no.	Latitude, [°S]	Longitude, [°E]	Date	Days after 1st / 2nd fertilization	Patch class.	N_2O ML conc.[a], [nmol L^{-1}]	N_2O ML sat.[a], [%]
513	49.59	2.05	28 Feb 04	16 / 2	In	13.3 ± 0.1 (5)	102 ± 1 (5)
514	49.31	2.34	29 Feb 04	17 / 3	Out	13.5 ± 0.3 (3)	104 ± 2 (3)
544	49.36	1.87	07 Mar 04	24 / 10	In	13.8 ± 0.5 (3)	106 ± 4 (3)
546	49.47	2.09	10 Mar 04	27 / 13	Out	13.1 (2)	102 (2)
570	49.43	2.05	14 Mar 04	31 / 17	In	13.1 ± 0.3 (5)	102 ± 3 (5)
580	49.12	2.38	16 Mar 04	33 / 19	In	12.5 ± 0.2 (3)	97 ± 1 (3)
586	49.50	2.10	18 Mar 04	35 / 21	Out	13.1 ± 0.5 (4)	102 ± 4 (4)

[a] Given as average ± standard deviation. Number of depths used for averaging is given in parenthesis.

The N_2O profiles showed a pronounced maximum between 500 and 750 m which was associated with the O_2 minimum and the nitrate maximum (Figure 1) indicating that nitrification was the main N_2O formation process. Our N_2O concentrations are comparable to N_2O measurements from the South Atlantic and Southern Oceans [*Butler et al.*, 1995; *Law and Ling*, 2001; *Rees et al.*, 1997].

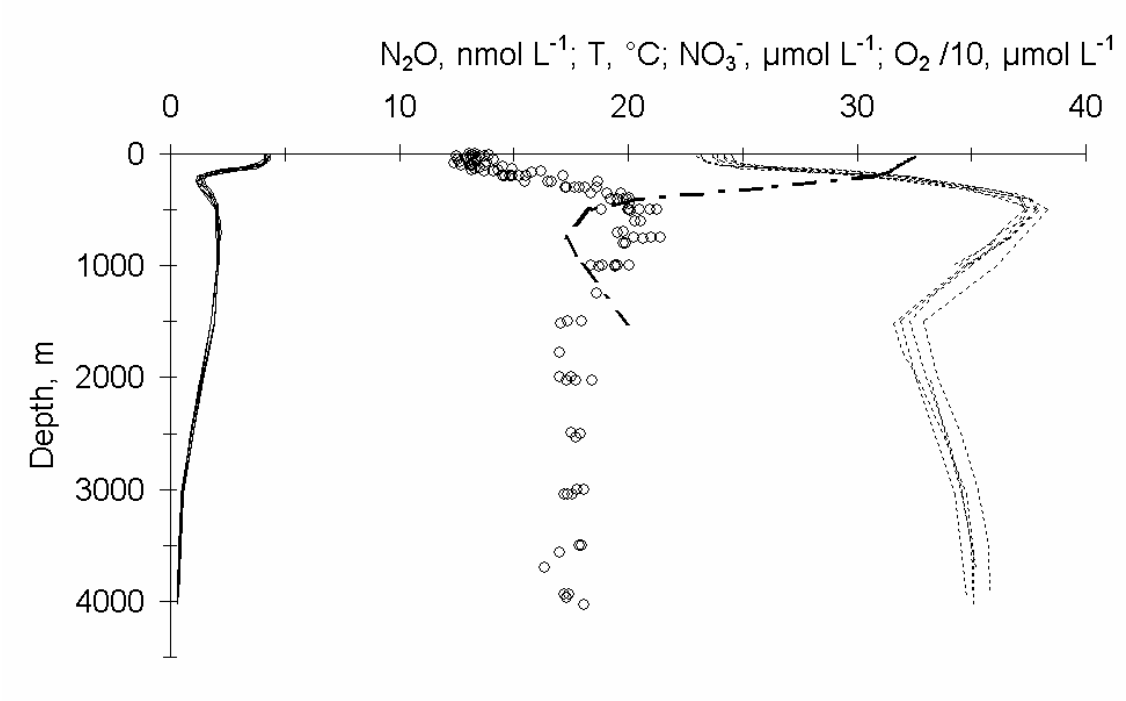

Fig. 1: N_2O (open circles), water temperature (solid lines), NO_3^- (dashed lines), and O_2 (dashed dotted line) at the EIFEX stations listed in Table 1. O_2 data are only available for station 570 in the depth range from 0-1500m. Please note that O_2 is given in µmol L^{-1} divided by 10.

Following the approach by *Law and Ling* [2001], we fitted a polynomial to the N_2O-σ_t data of stations 546 and 587 (Figure 2). Out-station 514 was excluded because it obviously was not representative as indicated by the data in Figure 2 (see also section EIFEX setting). A comparison of the N_2O concentrations of the in-stations with the polynomial fit based on the out stations revealed no significant differences (Figure 2).

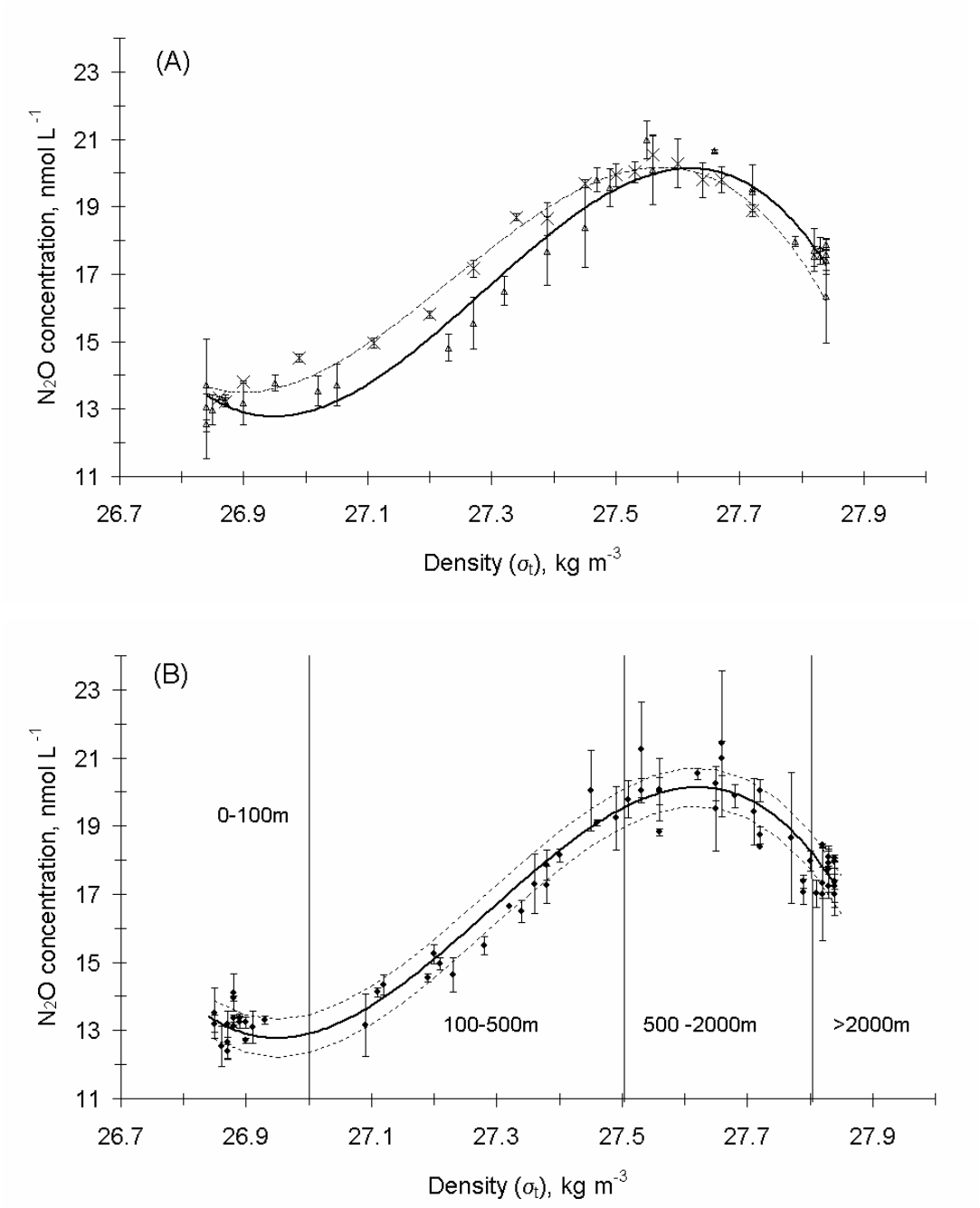

Fig. 2: N_2O concentrations vs. density (σ_t) during EIFEX. (A) Out-stations: Triangles stand for stations 546 and 587 and crosses stand for station 514. The bold solid line represents a 3rd order polynomial fit based on stations 546 and 587 (see text for statistical details). The thin solid line represents a 3rd order polynomial fit based on station 514. (B) In-stations: 513, 544, 570, and 580 (symbols) compared with the polynomial fit based on out-stations 546 and 587 (bold line, see panel A). The dashed lines indicate the standard error of the predicted N_2O. Depths intervals are indicated.

A 3rd order polynomial fit to the in-stations ($-52.766x^3 + 4320.7x^2 - 117{,}915x + 1{,}072{,}529$, $r^2 = 0.95$, $n = 67$, standard error of predicted $N_2O = \pm 0.63$ nmol L^{-1}) was almost identical to the out-stations' fit ($-48.474x^3 + 3967.8x^2 - 108{,}241x + 984{,}148$, $r^2 = 0.96$, $n = 30$, standard error of predicted $N_2O = \pm 0.56$ nmol L^{-1}). Thus, we conclude that no significant changes in the N_2O concentrations occurred during EIFEX.

Our conclusion is in contrast to the observation by *Law and Ling* [2001]. They found an accumulation of N_2O up to 0.9 – 1 nmol L^{-1} in the pycnocline (60 – 80 m water depth) within 13 days during SOIREE. Adapting a N_2O accumulation rate of 0.08 nmol L^{-1} d^{-1} (= 1 nmol L^{-1} / 13 days), an increase of 2.6 nmol L^{-1} (= 0.08 nmol L^{-1} x 33 days) would have been expected for a N_2O accumulation in the pycnocline in 100 – 200 m during EIFEX. This was not the case (Figure 2). It is possible that N_2O accumulation in the pycnocline was not detected because of insufficient analytical precision and/or coarse sampling of the depths profiles: A possible N_2O accumulation must have been low (< 0.5 nmol L^{-1} over the duration of the experiment as implied by our mean analytical error) or must have taken place in a narrow depth range of less than 40 m (i.e., the mean depth spacing of sampling from the surface to the pycnocline in about 200 m). Moreover, in contrast to EIFEX, Fe addition during SOIREE was performed four times within a week over a much smaller area (50 km^2, *Law and Ling* [2001]). Therefore, the observed N_2O accumulation in the pycnocline during SOIREE may have been a fast short-term response to the intensive short-term Fe fertilization. Because we started N_2O sampling 16 days after the first Fe addition (i.e., 2 days after the second Fe addition) we might have missed this short-term signal during EIFEX.

During EIFEX chlorophyll *a* (chl *a*) standing stocks increased 3 fold until day 26, but remarkably decreased thereafter [*Peeken et al.*, 2005]. The main beneficiaries of the iron fertilization were diatoms in all size classes [*Hoffmann et al.*, 2005]. Towards the end of the experiment, the diatom marker fucoxanthin and chl *a* could be followed down the water column to 4000 m and a low ratio of phaeopigments to chl *a* indicated the export of fresh material most likely originating from the iron fertilized patch [*Peeken et al.*, 2005]. An explanation for the absence of an increase of N_2O in the deep (e.g., in the O_2 minimum zone) might be the very rapid export of the fresh phytoplankton material to the deep ocean during EIFEX [*Peeken et al.*, 2005], which started about 23 days after the second Fe addition. Thus, we can argue that the rapid export of organic material during EIFEX might have been too rapid for the nitrifying bacteria in the deep ocean to adapt to

and, thus, an additional build-up of N2O in the deep could not take place. Nitrifying bacteria, especially ammonium-oxidizing bacteria (AOB), are known for lag phases up to several weeks after periods of low metabolic activities [*Schmidt et al.*, 1999].
The responsible process for the N_2O accumulation during SOIREE [*Law and Ling*, 2001] and the proposed further increase of N_2O in prolonged iron fertilization experiments could not be identified. Thus, a possible link between N_2O accumulation and Fe fertilization remains to be not a simple cause-and-effect mechanisms and the magnitude of a possible radiative offset still needs to be proven.

4. Conclusions

We did not observe a N_2O accumulation during the *in situ* iron fertilization experiment EIFEX in the subpolar South Atlantic Ocean in February/March 2004. This is in contrast to previous measurements by *Law and Ling* [2001] in the Australasian sector of the Southern Ocean. We conclude that Fe fertilization does not necessarily trigger additional N_2O formation, which might depend on differences of the environmental conditions (e.g., the fate of the Fe-induced phytoplankton bloom). We caution, therefore, that predictions of a radiative offset caused by a Fe-induced additional release of oceanic N_2O [*Jin and Gruber*, 2003; *Law and Ling*, 2001] might be overestimated. In order to solve this problem further long-term experiments with particular emphasis on sedimentation processes are necessary to prove a link between Fe addition and enhancement of N_2O formation and the subsequent release of N_2O to the atmosphere.

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Chapter 5

Nitrous oxide water column distribution during the transition from anoxic to oxic conditions in the Baltic Sea

Sylvia Walter, Ulrich Breitenbach, Hermann W. Bange, Günther Nausch, Douglas W.R. Wallace

Abstract

In January 2003, a major inflow of cold and oxygen-rich North Sea Water in the Baltic Sea terminated an ongoing stagnation period in parts of the central Baltic Sea. In order to investigate the role of North Sea Water inflow to the Baltic Sea with regard to the production of nitrous oxide (N_2O), we measured dissolved and atmospheric nitrous oxide at 26 stations in the southern and central Baltic Sea in October 2003.

At the time of our cruise, water renewal had proceeded to the eastern Gotland Basin, whereas the western Gotland Basin was still unaffected by the inflow. The deep water renewal was detectable in the distributions of temperature, salinity, and oxygen concentrations as well as in the distribution of the N_2O concentrations: Shallow stations in the Kiel Bight and Pomeranian Bight were well-ventilated with uniform N_2O concentrations near equilibrium throughout the water column. In contrast, stations in the deep basins, such as the Bornholm and the Gotland Deep, showed a clear stratification with deep water affected by North Sea Water. Inflowing North Sea Water led to changed environmental conditions, especially enhanced O_2 or declining H_2S concentrations, thus, affecting the conditions for the production of N_2O. Pattern of N_2O profiles and correlations with parameters like oxygen and nitrate differed between the basins. The dominant production pathway seems to be nitrification rather than denitrification.

No indications for advection of N_2O by North Sea Water were found. A rough budget revealed a significant surplus of in situ produced N_2O after the inflow. However, due to the permanent halocline, it can be assumed that the formed N_2O does not reach the atmosphere. Hydrographic aspects therefore are decisive factors determining the final release of produced N_2O to the atmosphere.

1. Introduction

1.1 Nitrous oxide

Nitrous oxide (N_2O) is an important atmospheric trace gas which influences, directly and indirectly, the Earth's climate: In the troposphere, it acts as a greenhouse gas with a relatively long atmospheric lifetime of 114 years [*Prather et al.*, 2001]. In the stratosphere it is the major source for nitric oxide radicals, which are involved in one of the main ozone reaction cycles [*WMO*, 2003].

N_2O is mainly formed during microbial processes such as nitrification and denitrification. Nitrification is an aerobic two-step process in which ammonium is oxidized to nitrate. In this process, in which typically two groups of bacteria are involved, N_2O is assumed to be a by-product, the exact metabolism however is still under discussion [*Ostrom et al.*, 2000]. In suboxic habitats, nitrate can be reduced by denitrification to molecular nitrogen, with N_2O as an intermediate [*Cohen and Gordon*, 1978]. N_2O may also be produced by coupled nitrification and denitrification at oxic/suboxic boundaries, due to the transfer of intermediates such as nitrate and nitrite [*Yoshinari et al.*, 1997]. Other possibilities are the production of N_2O during nitrifier-denitrification or aerobic denitrification [*Wrage et al.*, 2001]. Both processes enable nitrifiers to oxidize NH_4^+ to NO_2^-, followed by the reduction of NO_2^- to N_2O or N_2 [*Robertson and Kuenen*, 1984; *Robertson et al.*, 1988; *Richardson*, 2000]. In anoxic habitats N_2O is used, instead of oxygen, as an electron acceptor [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978].

The yield of N_2O during these processes strongly depends on the concentration of dissolved oxygen and nitrate [*Brettar and Rheinheimer,* 1991; *Goreau et al.,* 1980; *Vollack and Zumft,* 2001; *Wetzel*, 1983], with maximal N_2O accumulation at the interface between oxic and suboxic layers and depletion in anoxic layers [*Codispoti et al.*, 2005]. Positive correlations between N_2O and oxygen or nitrate are commonly interpreted as an indication of N_2O production by nitrification [*Yoshinari*, 1976; *Yoshida et al.*, 1989; *Cohen and Gordon*, 1978]. In contrast, production by denitrification is inferred by missing correlations [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978]. However, up to now the dominant production pathway for N_2O on the global scale remains unclear and is discussed controversially [*Codispoti et al.*, 2001; *Popp et al.*, 2002; *Yamagishi et al.*, 2005].

Oceans emit more than 25 % of natural produced N_2O and contribute significantly to the global N_2O budget [*Prather et al.*, 2001; *Seitzinger et al.* 2000]. Particularly coastal re-

gions, including estuarine and upwelling regions play a major role for the formation and release of N_2O to the atmosphere [*Bange et al.*, 1996; *Naqvi et al.*, 2000; *Seitzinger et al.*, 2000]. In the Baltic Sea, first investigations were made by *Rönner* [1983] who found the Baltic Sea to be a source of atmospheric N_2O. In contrast to open ocean areas coastal regions are expected to be more influenced by conversion processes in sediments or by riverine inputs. In the Bodden waters and Danish fjords of the Baltic Sea enhanced N_2O concentrations were correlated with seasonal riverine input [*Jørgensen and Sørensen*, 1985; *Dahlke et al.,* 2000]. Additionally, denitrification processes in sediments were shown to contribute to the release of N_2O in Danish fjords [*Jørgensen and Sørensen*, 1985].

1.2 Study area

Samples of dissolved N_2O were measured at 26 stations in the western, southern and central Baltic Sea. The cruise took place on board the German research vessel Gauss (expedition no. 11/03/04) from 13th October to 25th October 2003 as part of the Cooperative Monitoring in the Baltic Sea Environment (COMBINE) program of the Baltic Marine Environment Protection Commission (Helsinki Commission, HELCOM, see http://www.helcom.fi). The locations of sampled stations are shown in Fig. 1.

The Baltic Sea is a mediterranean sea of the Atlantic Ocean and part of the European continental shelf. It consists of a series of basins (Arkona, Bornholm, and Gotland Basin; see Fig. 1), with restricted horizontal and vertical water exchange due to shallow sills and a clear salinity stratification of water masses.

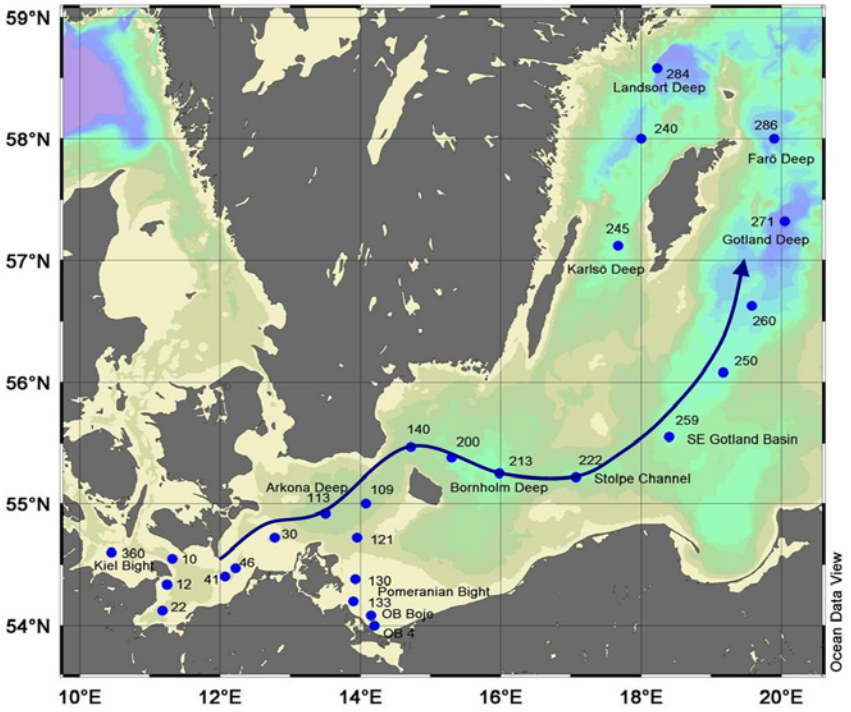


Fig. 1: Map of the western, southern and central Baltic Sea with locations of the stations. The stations were grouped as follows: well-mixed stations are number 10, 12, 22, 30, 41, 46, 121, 130, 133, 360, OB Boje and OB 4; the Arkona Basin is represented by station 109 and 113; the Bornholm Basin is represented by station 140, 200, 213 and 222; in the eastern Gotland Basin station 250, 259, 260, 271 and 286 were grouped; and the western Gotland Basin is represented by station 240, 245 and 284. The arrow indicates the main flow direction of North Sea Water.

In January 2003 a major inflow of cold, highly saline and oxygen-rich North Sea Water was observed. It was the most important inflow event since 1993 and terminated the ongoing stagnation period in southern parts of the central Baltic Sea [*Feistel et al.*, 2003; *Nausch et al.*, 2003]. This inflow event was preceded by a minor inflow of warmer and less oxygenated water in August 2002. Due to the inflow of North Sea Water oxygen conditions changed from anoxic to oxic in most parts of the Baltic Sea. From the inflow in January 2003 until our cruise in October 2003 water renewal was already detectable at the Farö Deep (# 286), however the western Gotland Basin was still unventilated [*Feistel et al.*, 2003; *Nausch et al.*, 2003].

Due to the fact that N_2O production highly depends on environmental conditions such as e.g. oxygen concentration [e.g., *Naqvi et al.*, 2000] any natural or anthropogenic-induced shifts of coastal ecosystems will modulate the formation and subsequent release of N_2O to the atmosphere. In this context the inflow of North Sea Water into the Baltic Sea offered a good opportunity to investigate naturally changing environmental conditions with regard to the production of N_2O.

1.3 Definition of water masses

We refer to four different water masses, characterized by temperature, salinity and oxygen concentrations (Fig. 3). The definition of water masses follows the description of the ´Institut für Ostseeforschung` (IOW) cruise reports [*Nausch,* 2003a; *Nagel,* 2003; *Feistel,* 2003; *Nausch,* 2003b; *Nausch,* 2003c; *Wasmund,* 2003] and the hydrographic-chemical report of the Baltic Sea in 2003 [*Nausch et al.,* 2004].

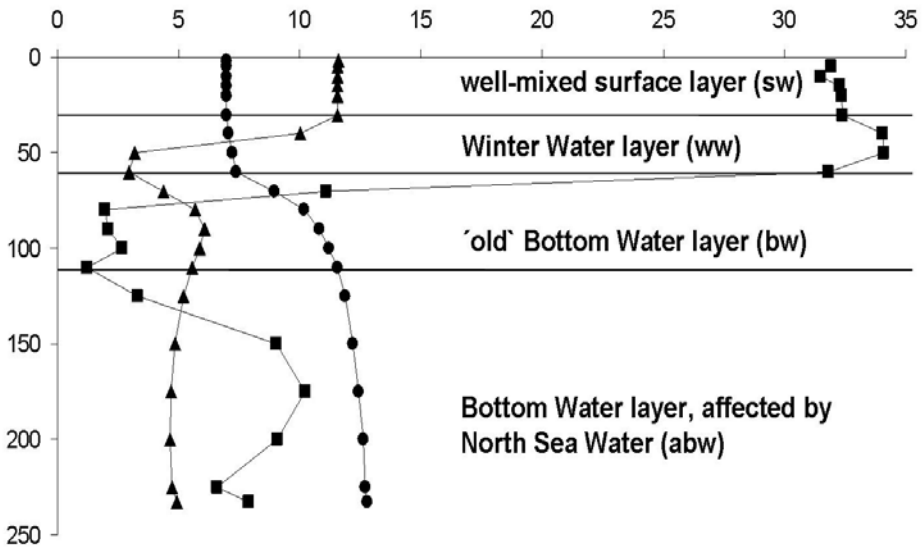


Fig. 2: Characterization of different water masses in the Baltic Sea, for example at station 271 in the Eastern Gotland Basin (triangles: temperature [°C], circles: salinity, squares: oxygen [µmol 10^1 L^{-1}]

The Surface Water layer (sw) was characterized by uniform temperature and salinity, in combination with high oxygen concentrations. Below this layer, rapidly decreasing temperatures indicated Winter Water (ww), which is formed annually during convection in winter. Salinity and oxygen concentrations were still uniform. The ´old` Bottom Water (bw) was visible by increasing temperature and simultaneously increasing salinity. In this water mass, located below the Winter Water, oxygen concentrations decreased rapidly, to anoxic conditions at some stations. A permanent halocline between Winter Water and Bottom Water strongly restricts the vertical exchange and is the reason for the development of stagnant deep waters with oxygen depletion up to anoxia accompanied by accumulation of hydrogen sulphide (H_2S). Bottom Water, affected by the North Sea Water inflow in January 2003 (abw) was characterized by decreasing temperature and enhanced oxygen concentrations compared to previous Bottom Water (bw) values. Due to its higher density the affected Bottom Water lifts up the ´old` Bottom Water.

2. Methods

Water samples for N_2O analysis were collected in triplicate from various depths, taken with a 24-Niskin-bottle rosette, equipped with a CTD-probe. The analytical method applied was a modification of the method described by [*Bange et al.*, 2001]. Bubble free samples were taken immediately following oxygen sampling from the rosette in 24 mL glass vials, sealed directly with butyl rubber stoppers and crimped with aluminium caps. To prevent microbial activity, samples were poisoned with 500 µL of a 2 mM mercury chloride solution.10 mL of the sample were then replaced with a helium headspace for each vial, and the samples were equilibrated for at least two hours at room temperature (temperature was recorded continuously). A 9 mL subsample from the headspace was used to flush a 2 mL sample loop after passing through a moisture trap (filled with Sicapent®, Merck Germany). Gaschromatographic separation was performed at 190 °C on a packed molecular sieve column (6 ft x 1/8" SS, 5 A, mesh 80/100, Alltech GmbH, Germany). The N_2O was detected with an electron capture detector. A mixture of argon with 5 percent by volume methane was used as carrier gas with a flow of 21 mL min^{-1}. For the two-point calibration procedure we used standard gas mixtures with 311.8 ± 0.2 ppb and 346.5 ± 0.2 ppb N_2O in synthetic air (Deuste Steininger GmbH, Mühlhausen Germany). The standard mixtures have been calibrated against the NOAA (National Oceanic and Atmospheric Administration, Boulder, Co.) standard scale in the laboratories of the Air Chemistry Division of the Max Planck Institute for Chemistry, Mainz, Germany.

2.1 Calculations

N_2O water concentrations (C_{N2O}) were calculated as follows:

$$C_{N2O}\left[\text{nmol L}^{-1}\right] = \left(\beta \text{ x P } V_{wp} + \frac{\text{x P}}{\text{R T}} V_{hs} \right) / V_{wp} \qquad (1)$$

where β stands for the Bunsen solubility in nmol L^{-1} atm^{-1} [*Weiss and Price*, 1980], x is the dry gas mole fraction of N_2O in the headspace in ppb, P is the atmospheric pressure in atm, V_{wp} and V_{hs} stand for the volumes of the water and headspace phases, respectively. R is the gas constant (8.2054 10^{-2} L atm mol^{-1} K^{-1}) and T is the temperature during equilibration. The salinity was measured by the CTD-Sensor during water sample collec-

tion; the temperature was measured while subsampling the headspace of the sample vial (i.e. the equilibration temperature). The overall relative mean analytical error was estimated to be ± 1.8 %.

The excess N_2O (ΔN_2O) was calculated as the difference between the calculated N_2O equilibrium concentration and the measured concentration of N_2O as follows

$$\Delta N_2O\ [\text{nmol L}^{-1}] = N_2O\ (\text{observed}) - N_2O\ (\text{equilibrium}). \qquad (2)$$

Since the water masses in the Baltic Sea are comparably young (e.g. 11 years for the oldest bottom water at the Landsort Deep) [*Meier*, 2005] it is reasonable to calculate the equilibrium value with the actual atmospheric N_2O mole fraction. During the cruise we measured a mean of 318 ppb (± 3 ppb, n = 84) in the atmosphere, which is in good agreement with the monthly mean of 318.5 ±0.2 ppb in October 2003 measured at Mace Head, Ireland. This value was taken from the Advanced Global Atmospheric Gases Experiment (AGAGE) data set (updated version from May 2005, available at ftp://cdiac.esd.ornl.edu (subdirectory pub/ale_gage_Agage/Agage/gc-md/monthly) at the Carbon Dioxide Information Analysis Center in Oak Ridge, Tennessee).

The apparent oxygen utilization (AOU) was calculated as follows:

$$AOU\ [\mu\text{mol L}^{-1}] = O_2\ (\text{equilibrium}) - O_2\ (\text{observed}). \qquad (3)$$

The equilibrium values of dissolved oxygen (O_2) were calculated with the equation given by [*Weiss*, 1970]. The concentration of H_2S is expressed as the negative oxygen equivalent (1 µmol L^{-1} H_2S = 2.62 µmol L^{-1} O_2). Dissolved nutrients and CTD data were provided by the participating working groups.

The N_2O inventory of single basins m_{N2O} was calculated as follows:

$$m_{N2O}\ [\text{tons}] = \overline{C}_{N2O} * n_{N2O} * V * 10^{-3} \qquad (4)$$

where $\overline{C}_{N2O}$ is the mean measured N_2O concentration in the single basins from the upper halocline to the bottom [nmol L^{-1}], n_{N2O} is the mole weight of N_2O (44 g mol^{-1}) and V is the water volume of the single basins [km^3].

The water volumes are based on data published in chapter 4.4.1 [*HELCOM*, 1996], available at: www.vtt.fi/inf/baltic/balticinfo/index.html.

The N_2O content of basins was calculated with data of the following stations: Bornholm Basin: station 140, 200, 213, 222, eastern Gotland Basin: station 250, 259, 260, 272, western Gotland Basin: station 240, 245, 284. Station 286 is located in the northern part of the Gotland Basin and thus has not been taken into account.

Nitrification rates [N] were estimated for the Bornholm Basin and the eastern Gotland Basin.

$$N\left[\text{nmol } L^{-1}\ d^{-1}\right] = \frac{\Delta m_{N2O}}{d_{basin} * V_{basin} * n * 10^{-9}} * r_{N2O} \qquad (5)$$

where Δm_{N2O} is the difference of calculated N_2O content of the basins before and after the inflow event in tons, d_{basin} is the number of days from the first observation of the intrusion of North Sea Water untill our measurements (assumed by data of the cruise reports [*Nausch,* 2003a; *Nagel,* 2003; *Feistel,* 2003; *Nausch,* 2003b; *Wasmund,* 2003; *Nausch,* 2003c]).

V_{basin} is the calculated volume of the basins [km^3] (based on data published in chapter 4.4.1 [*HELCOM*, 1996], available at: www.vtt.fi/inf/baltic/balticinfo/index.html), n is the mole weight of N_2O (44 g mol^{-1}), and r_{N2O} is the assumed N_2O release of 0.3 % in continental shelves during nitrification [*Seitzinger and Kroeze,* 1998].

3. Results

In order to account for the hydrographic characteristics of the Baltic Sea and the direction of the inflow of North Sea Water, we present the results according to the following classifications: I) well-mixed basins such as the Kiel, Lübeck and Pomeranian Bights and II) clearly stratified basins such as the Arkona, the Bornholm, the western and the eastern Gotland Basin (see Fig. 2). For each basin selected profiles are shown.

3.1 Well-mixed basins

At shallow stations, with depths < 30 m (station 10, 12, 22, 30, 41, 46, 121, 130, 133, 360, OB Boje, OB 4, Fig. 1), water masses were well mixed, and profiles showed nearly uniform vertical distributions of all parameters (Fig. 3a). Concentrations of N_2O were

near equilibrium; however the Pomeranian Bight (station 130, 133, OB Boje, OB 4) showed enhanced saturation values (104.6 ± 7.9 %) in comparison with the Kiel Bight (station 360) and the Lübeck and Mecklenburg Bight (station 10, 12, 22, 41, 46; 79.3 ± 10.7 %). No correlations were found between ΔN_2O and other parameters like O_2 and NO_3^- (Fig. 3b-c).

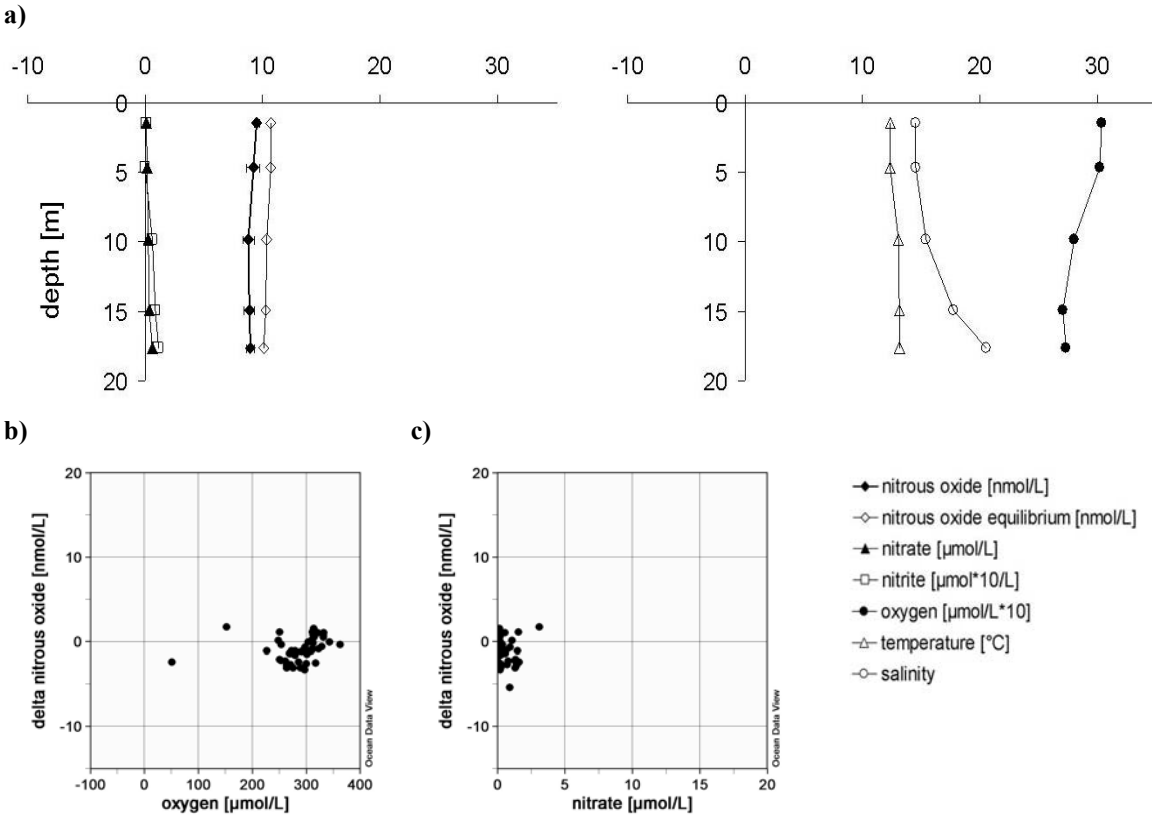


Fig. 3: well mixed basins; a) left plot with profiles of N_2O, calculated N_2O equilibrium concentration, NO_3^-, NO_2^- at station 41 in the Mecklenburg Bight and right plot with profiles of temperature, salinity and oxygen at station 41 in the Mecklenburg Bight; b) ΔN_2O plotted against oxygen at all stations < 30 m; c) ΔN_2O plotted against NO_3^- at all stations < 30 m

3.2 Stratified basins

Basins with water depths > 30 m (Fig. 4-7) were clearly stratified into layers of well mixed Surface Water (sw), Winter (ww) and Bottom Water (bw) as described above. At several stations Bottom Water was affected by North Sea Water (abw), up to the Farö Deep in the northern part of the central Baltic Sea (Fig. 1, station 286) [*Feistel et al.*, 2003]. However, below 110 m the deep water of the Farö Deep was still anoxic, though with decreasing H_2S concentrations from 125 m to the bottom (Fig. 6a, lower profiles). Stations in the western Gotland Basin such as the Landsort Deep (station 284, Fig. 7a) or

the Karlsö Deep (station 245, not shown) were still unaffected by the inflow event, thus below 80 m H_2S concentrations were uniform.

3.2.1 Arkona Basin

In the Arkona Basin (stations 109 and 113 (Fig. 4a)), N_2O concentrations were constant and near equilibrium (10.9 ± 0.7 nmol L^{-1}) throughout the water column. In the Winter Water below the thermocline at 15 m O_2 concentrations decreased, associated with increasing NO_2^-, NO_3^- and NH_4^+. ΔN_2O was slightly negatively correlated with O_2 (Fig. 4b), and positively correlated with NO_3^- (Fig. 4c). At the bottom below 40 m inflowing North Sea (arrow in Fig. 4a) water formed a 5 to 10 m thick oxygen enriched layer, however with no clear influence on the N_2O concentration.

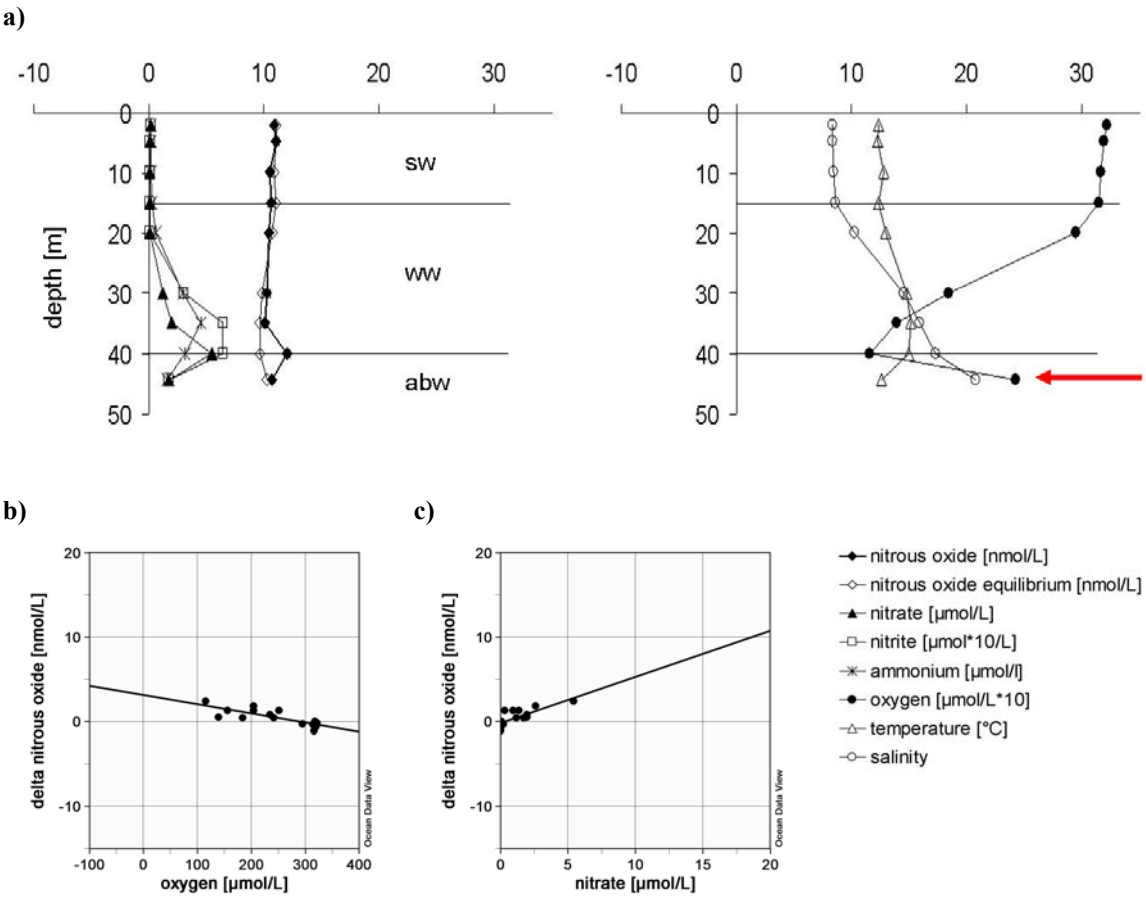


Fig. 4: Arkona Basin; a) station 113 (Arkona Deep): left plot with profiles of N_2O, N_2O equilibrium concentration, NO_3^-, NO_2^-, right plot with profiles of temperature, salinity and oxygen, the arrow indicate the influence of North Sea Water; abbreviations see Fig. 2.; b) ΔN_2O plotted against oxygen (at all stations in the Arkona Basin, $y = -0.011 x + 3.132$, $R^2 = 0.67$); c) ΔN_2O plotted against NO_3^- (at all stations in the Arkona Basin, $y = 0.546 x - 0.807$, $R^2 = 0.66$)

3.2.2 Bornholm Basin

In the Bornholm Basin (Fig. 5, stations 140, 200, 213 and 222), N_2O profiles in the central basin (stations 200 (not shown) and 213 (Fig. 5a)) can be clearly distinguished from stations where water flows into and out of the basin. At station 140 (inflow, not shown) concentrations and distribution of N_2O and ΔN_2O were comparable to the Arkona Basin. At station 222 (outflow, not shown) N_2O concentrations in the surface layer were uniform near equilibrium at approximately 10 nmol L^{-1}, below the surface layer concentrations were uniform around 15.4 nmol L^{-1}. In the central Bornholm Basin, at station 200 (not shown) and 213 (Fig. 5a) N_2O concentrations increased rapidly within the layer affected by North Sea Water (abw, below 60 m), with N_2O values up to 31.3 nmol L^{-1} (station 200). These were the highest values measured during the entire cruise. In water masses above, N_2O was near equilibrium, with slightly enhanced ΔN_2O values in the ´old` Bottom Water (bw, 40 – 60 m). In the Bornholm Basin ΔN_2O was clearly negatively correlated with oxygen and positively with NO_3^- (Fig. 5b-c), however, both correlations were nonlinear and were fitted best by polynomials.

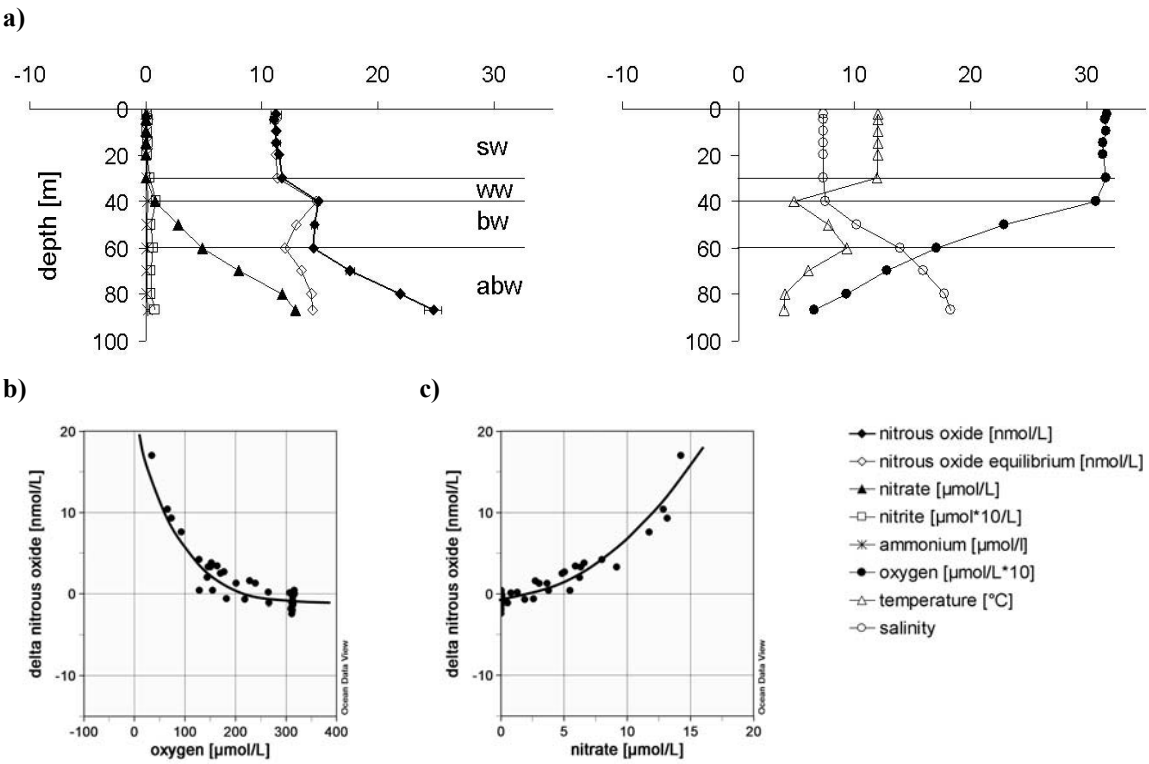
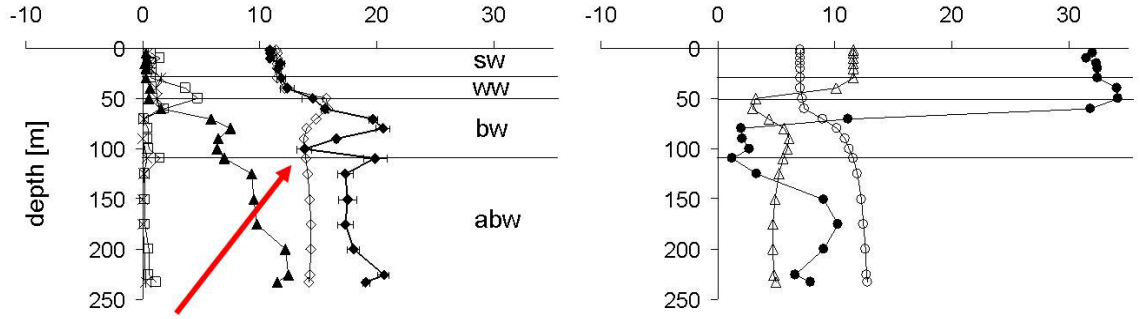


Fig. 5: central Bornholm Basin; a) station 213 (Bornholm Deep): left plot with profiles of N_2O, N_2O equilibrium concentration, NO_3^-, NO_2^-, right plot with profiles of temperature, salinity and oxygen, abbreviations see Fig. 2; b) ΔN_2O plotted against oxygen (at all stations in the Bornholm Basin, $y = 0.0003\ x^2 - 0.1531\ x + 19.517$, $R^2 = 0.88$); c) ΔN_2O plotted against NO_3^- (at all stations in the Bornholm Basin, $y = 0.0585\ x^2 + 0.1438\ x - 0.6155$, $R^2 = 0.90$)

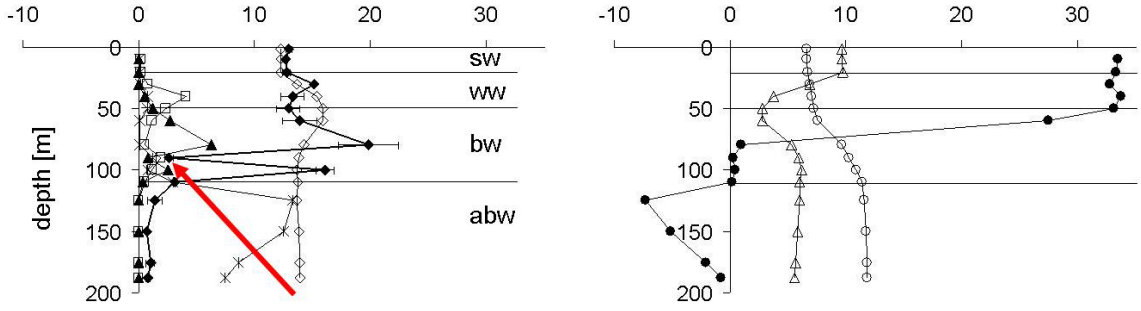
3.2.3 Eastern Gotland Basin

The situation became more complex in the eastern Gotland basin (stations 259, 250, 260, 271 and 286). Profiles were not as homogeneous as in the Arkona or Bornholm Basin. Again, N_2O concentrations were near equilibrium in the surface layer (sw, 0 - 20/30 m) and the Winter Water (ww, 20/30 – 60 m). At station 271 (Fig. 6a, upper profiles) the Bottom Water (bw) was completely oxygenated, with N_2O values at approximately 20 nmol L^{-1} and positive ΔN_2O. At station 286 (Fig. 6a, lower profiles) the Bottom Water (bw) was affected by the North Sea Water too, but was still anoxic. Inflow of North Sea Water was detectable by decreasing H_2S concentrations down to the bottom. Throughout the Bottom Water N_2O concentrations remained near zero. At station 250 (not shown), 271 (Fig. 6a, upper profiles) and 286 (Fig. 6a, lower profiles) a sharp local minimum of N_2O concentrations was observed at depths between 90 and 110 m (see arrows in Fig. 6a), combined with a local minimum in NO_3^- values. Except for the anoxic water masses, ΔN_2O was linearly correlated with O_2 and NO_3^- (Fig. 6b-c).

a) station 271 (Gotland Deep)



station 286 (Farö Deep)



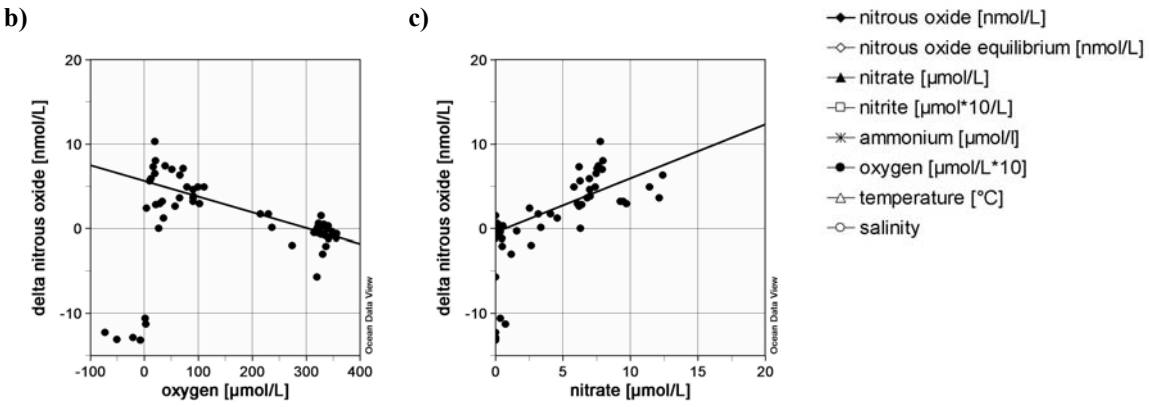
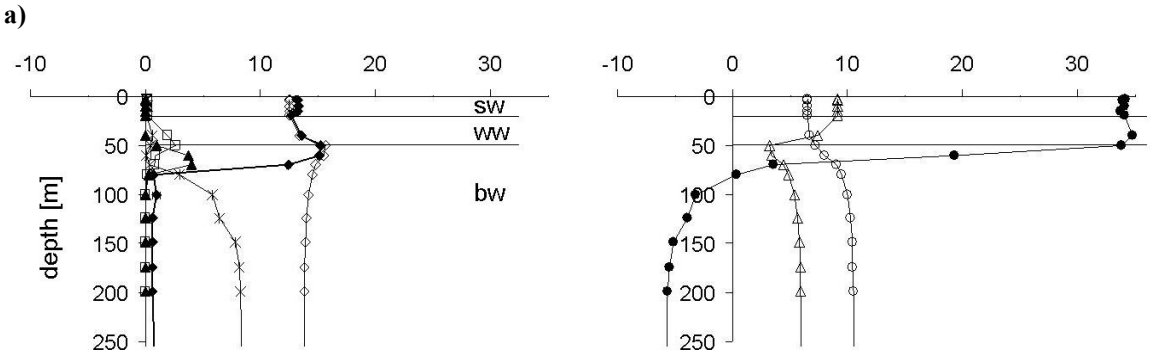


Fig. 6: Eastern Gotland Basin; a) station 271 (Gotland Deep, upper plots) and 286 (Farö Deep, lower plots): left plots with profiles of N_2O, N_2O equilibrium concentration, NO_3^-, NO_2^-; right plots with profiles of temperature, salinity and oxygen, abbreviations see Fig. 2; b) ΔN_2O plotted against oxygen (at all stations in the Eastern Gotland Basin, $y = -0.019\ x + 5.625$, $R^2 = 0.67$ (except for $O_2 < 3$ µmol L^{-1})); c) ΔN_2O plotted against NO_3^- (at all stations in the Eastern Gotland Basin, $y = 0.639\ x - 0.459$, $R^2 = 0.62$ (except for $O_2 < 3$ µmol L^{-1}))

3.2.4 Western Gotland Basin

The western Gotland Basin with stations 284 (Fig. 7a), 245 and 240 revealed the "old" conditions, showing characteristics as yet unaffected by the latest intrusion of oxic North Sea Water. N_2O in the surface layer (sw, 0 - 20/40 m) and Winter Water (ww, 20/40 – 60 m) was near equilibrium. Below 50 m, oxygen concentrations decreased rapidly and N_2O concentrations dropped sharply within the oxic/anoxic interface and remained near zero in the anoxic deep waters. ΔN_2O values were negative and were not correlated with NO_3^- (Fig. 7c). ΔN_2O was logarithmically correlated with oxygen (Fig. 7b).



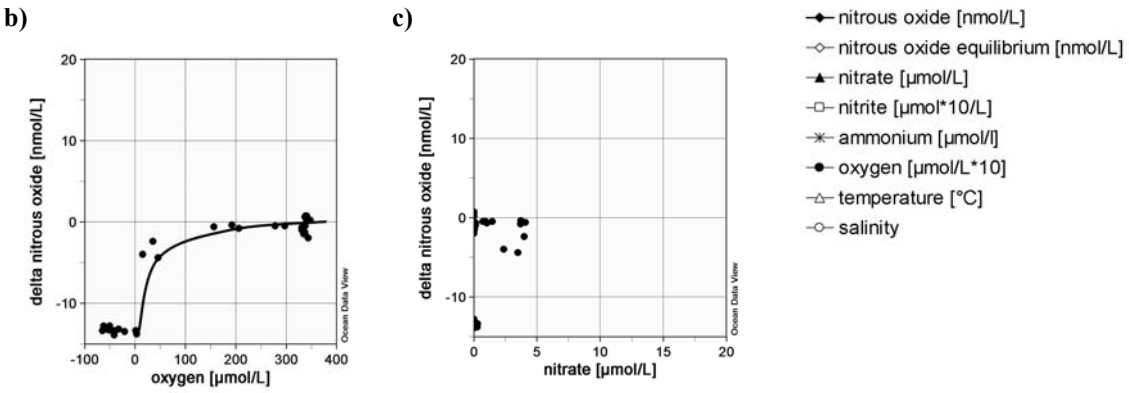


Fig. 7: Western Gotland Basin; a) station 284 (Landsort Deep): left plot with profiles of N_2O, N_2O equilibrium concentration, NO_3^-, NO_2^-, right plot with profiles of temperature, salinity and oxygen, abbreviations see Fig. 2; b) ΔN_2O plotted against oxygen (at all stations in the Western Gotland Basin; $y = 2.2467 \text{ Ln } (x) - 13.322$, $R^2 = 0.86$, (with exception of $O_2 < 0$ µmol L^{-1})) c) ΔN_2O plotted against NO_3^- (at all stations in the Western Gotland Basin)

3.3 Estimated contribution of the North Sea Water inflow to the production of N_2O

The North Sea Water inflow consisted of a water volume of 200 km^3 [*Feistel and Nausch,* 2003]. With an assumed N_2O concentration of 10 ± 2 nmol L^{-1} [*Law and Owens*, 1990], the North Sea Water transported approximately 88 ± 18 tons N_2O into the Baltic Sea.

Before the North Sea Water inflow, the deep waters below the halocline were anoxic, not only in the western but also in the eastern Gotland Basin and the Bornholm Basin [*Schmidt*, 2002]. Thus, N_2O concentrations near zero similar to measured profiles in the western Gotland Basin in October 2003 (Fig. 7a) can be assumed. This is supported by the drop in concentration at station 286 (Fig. 7a, lower profile), which is assumed to be related to the previously anoxic bottom water. The mean N_2O concentration in the western Gotland Basin was 0.97 ± 0.34 nmol L^{-1}, on the basis of these values the calculated N_2O content of the Bornholm Basin, the eastern and the western Gotland Basin was approximately 92 ± 33 tons before the inflow.

After the inflow event the Bornholm Basin and the eastern Gotland Basin are clearly influenced by the North Sea Water, whereas the western Gotland Basin was still unaffected [*Nausch,* 2003a; *Nagel,* 2003; *Feistel,* 2003; *Nausch,* 2003b; *Wasmund,* 2003; *Nausch,* 2003c]. The N_2O content of the basins, calculated with the mean of measured N_2O concentrations below the halocline in these basins, was about 1222 ± 266 tons (Table 1).

Table 1: Estimated N_2O content of single basins in the Baltic Sea below the halocline, before and after the inflow of North Sea Water in January 2003

	mean N_2O conc. below the halocline [nmol L^{-1}]	Water volume [km^3]	N_2O content before the inflow event [tons]	N_2O content after the inflow event [tons]
Bornholm Basin	> 50 m 16.59 ± 5.61	306	13 ± 5	223 ± 76
eastern Gotland Basin	> 70 m 18.46 ± 3.43	1195	51 ± 18	971 ± 180
western Gotland Basin	> 70 m 0.97 ± 0.34	657	28 ± 10	28 ± 10
Σ		2158	**92 ± 33**	**1222 ± 266**

4. Discussion

Over the past two decades the previously frequent inflows of North Sea Water became rather rare [*Feistel and Nausch,* 2003], and oxygen levels in deep waters decreased. Thus, oxygen conditions in the Baltic Sea deep water cover a continuum from permanently oxic (i.e. Arkona Basin) to almost permanently anoxic conditions (i.e. western Gotland Basin), with changes at non-regular intervals between anoxic and oxic (i.e. Bornholm Basin, eastern Gotland Basin) [*Feistel,* 2003; *Nausch,* 2003a; *Nausch,* 2003b; *Nausch,* 2003c; *Nagel,* 2003; *Wasmund,* 2003].

The inflow event in January 2003 rapidly changed the environmental conditions of the deep basins. With respect to the oxygen dependent production of N_2O, our measured N_2O concentrations reflect the continuum of unaffected and changing oxygen conditions quite well. In oxic and well mixed waters, vertical N_2O profiles were homogenous, with concentrations near equilibrium (Fig. 3a). Anoxic deep water layers, unaffected by North Sea Water (i.e. in the western Gotland Basin), had N_2O concentrations near zero (Fig. 7a). Therefore, in both cases no correlations between N_2O and either oxygen or nitrate were found (Fig. 3b-c, Fig. 7b-c). In contrast, stratified and recently ventilated water bodies in the Bornholm and eastern Gotland Basin revealed N_2O distributions that were clearly correlated with oxygen and nitrate (Fig.5b-c, Fig. 6b-c).

These vertical N_2O distributions are in general agreement with the few previously published N_2O profiles from the central Baltic Sea [*Rönner*, 1983; *Rönner and Sörensson*, 1985; *Brettar and Rheinheimer*, 1992]. However, the past environmental settings of the deep central Baltic Sea basins were different: N_2O profiles from the central Baltic Sea reported by [*Rönner*, 1983] were measured when oxic conditions prevailed during Au-

gust-September 1977 after a strong inflow event in 1976/1977 [*Schinke and Matthäus*, 1998]. Their N_2O profiles are comparable to our profiles, measured in the completely oxygenated Bornholm Basin during October 2003 (Fig. 5a). Anoxic conditions were re-established in July 1979 and May-June 1980. The shape of the N_2O profiles from the then anoxic Gotland Deep, measured by *Rönner and Sörensson* [1985] is comparable to the N_2O profiles measured in the western Gotland Basin (e.g., the Landsort Deep, Fig. 7a). This is the same for profiles measured by *Brettar and Rheinheimer* [1991] in August 1986 and July 1987 during the 1983-1993 stagnation periods [*Schinke and Matthäus*, 1998].

In the following sections we discuss the processes that may cause the observed distributions of N_2O in the different basins.

4.1 Non-biological aspects

In surface layers and well-mixed water bodies of shallow stations, observed N_2O concentrations were near the equilibrium due to exchange with the atmosphere. In the Winter Water N_2O concentrations were also near equilibrium, however with higher absolute values than in the surface layer (see Fig. 5a-7a). Mainly hydrographic aspects were here responsible for the observed N_2O distribution. This water mass is formed during winter convection, when N_2O concentrations were in equilibrium with the atmosphere and this signal is conserved during stratification of the upper layer in summer. The lower temperature and hence higher N_2O solubility during formation of the Winter Water are the reason for the enhanced N_2O concentrations in this layer.

A non-biological factor affecting N_2O in the deep water of the Baltic Sea might be advection with inflowing North Sea Water. Intrusion of N_2O by North Sea Water should be detectable at stratified stations, where the inflow of North Sea Water was clearly identified. In the Arkona Basin (station 109 and 113) this inflow was detectable at the bottom by lower temperature and higher oxygen concentrations; however, N_2O concentrations did not increase and remained close to equilibrium (Fig. 4a-b)**.** These results point to only low advection of N_2O by North Sea Water, and are supported by measurements of [*Law and Owens*, 1990]. They found N_2O concentrations close to equilibrium up to approximately 10 nmol L^{-1} in the North Sea. Thus, the enhanced N_2O values detected in layers affected by North Sea Water, for example in the Bornholm Basin (station 200 and 213), must originate from biological *in situ* production since the inflow, rather than advection.

4.2 Biological aspects

Previous studies demonstrated the existence of N_2O producing bacteria and investigated the biological pathways, namely nitrification and denitrification in the Baltic Sea [*Bauer*, 2003; *Brettar and Höfle*, 1993; *Brettar et al.*, 2001]. Both processes are commonly inferred by correlations between N_2O and oxygen or nitrate [*Yoshinari*, 1976; *Yoshida et al.*, 1989; *Cohen and Gordon*, 1978; *Butler et al.*, 1989].

4.2.1 Anoxic waters

In general, in anoxic and H_2S containing bottom waters N_2O concentrations were constantly near zero, and therefore no correlation with either O_2 or NO_3^- was found. The N_2O production by nitrification and denitrification might probably be inhibited by the presence of H_2S [*Joye and Hollibaugh*, 1995; *Knowles,* 1982, *Sørensen et al.,* 1980], and while changing to anoxic conditions, N_2O can be consumed during denitrification as an electron acceptor instead of oxygen [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978]. However, in contrast to other authors [*Rönner et al.*, 1983; *Brettar and Rheinheimer*, 1992] we found low and uniformly distributed concentrations of N_2O (up to 1.7 nmol L^{-1}) in the anoxic water masses, which may have been residuals of a previous production process during oxic conditions.

4.2.2 Suboxic waters

In suboxic waters and at the boundary to anoxic water masses N_2O is expected to be mainly produced by denitrification processes [*Codispoti et al.*, 2001], usually indicated by decreasing NO_3^- concentrations and a secondary NO_2^- peak [*Wrage et al.*, 2001; *Kristiansen and Schaanning*, 2002]. These indicators for denitrification were found only at the Farö Deep (station 286, 90m). However, no accumulation of N_2O was observed, rather a local minimum of N_2O was found (Fig. 6a, indicated by arrows). *Hannig et al.* [2005] recently investigated denitrification associated microorganisms in the Gotland Basin (station 271 and 286) in October 2003. They did not find denitrification activities in suboxic water masses, but a high denitrifying potential restricted to a narrow depth range at the oxic-anoxic interface and the sulfidic zone. However, in these depths an accumulation of N_2O was not found either.

The local minimum of N_2O was observed not only at the Farö Deep, but also at the Gotland Deep (Fig. 6a, indicated by arrows) and station 250 (profile not shown). A residual signal of the small inflow event in August 2002 could be observed at these depths be-

tween 90 and 110 m [*Feistel et al.*, 2003]. We assume that this minimum of N_2O is a previous signal of former anoxic bottom water, pushed up by the small inflow event in August 2002. The restriction of denitrification activity to a narrow depth range at anoxic-oxic boundaries was not only reported by *Hannig et al.* [2005] but also by *Brettar et al.* [2001]. Therefore, the lack of denitrification signals leads to the question, which processes might cause the measured N_2O concentrations.

4.2.3 Correlation between N_2O and O_2

In general, in oxic waters N_2O is positively correlated with nitrate, negatively with oxygen, indicating a production by nitrification. However, below a distinct threshold of oxygen concentration a clear inversion of relationship was found. Figure 8 shows the correlation between ΔN_2O and O_2 in the Baltic Sea. At O_2 concentrations > 50 µmol L^{-1} ΔN_2O is clearly negatively correlated with O_2, indicating production by nitrification (see Fig. 8, green data points). At O_2 concentrations < 20 µmol L^{-1} ΔN_2O and O_2 were significantly positively correlated (see Fig. 8, red data points), data between 20 µmol L^{-1} and 50 µmol L^{-1} were extremely scattered (see Fig. 8, black data points).

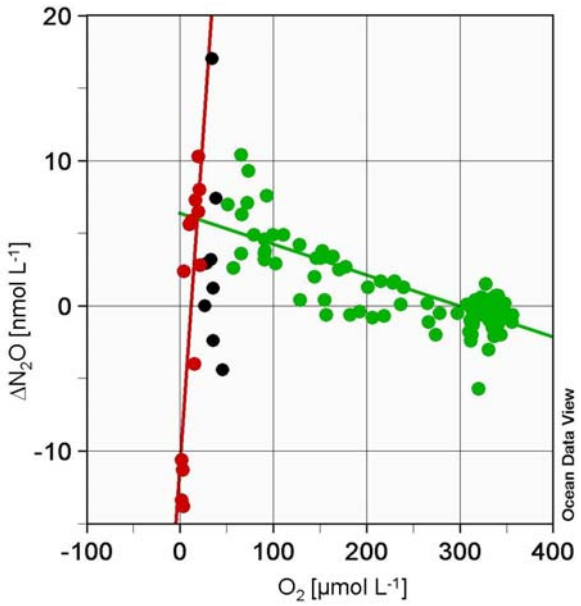


Fig. 8. Correlation between ΔN_2O and O_2 in the Baltic Sea. Correlations were calculated for oxic waters with O_2 concentrations > 50 µmol L^{-1} (green coloured, $y = -0.019\ x + 5.41$, $R^2 = -0.70$) and < 20 µmol L^{-1} (red coloured, $y = 1.038\ x - 11.36$, $R^2 = 0.81$). These concentrations were empirically tested and gave the best fittings for both correlations.

These findings suggest a change in N_2O converting processes. Particularly in environments with rapidly changing conditions it is advantageous for microorganisms to be able to switch between different metabolic pathways. The change between aerobic and an-

aerobic metabolism and thus the yield of N_2O during these processes is probably controlled particularly by the O_2 concentration, although little is known about the detailed mechanisms [*Baumann et al.*, 1996; *John*, 1977; *Sørensen*, 1987]. Our results suggest a production of N_2O during nitrification until an oxygen threshold of around 20 - 50 µmol L^{-1}, whereas the exact concentration is not to be determined due to the scattered data. Below this threshold N_2O seemed to be degraded; probably used as an electron acceptor instead of oxygen and thereby reduced to N_2 [*Elkins et al.*, 1978; *Cohen and Gordon*, 1978]. In the literature, threshold values of 2 µmol L^{-1} for nitrification are reported [*Carlucci and McNally*, 1969; *Gundersen et al.*, 1966]. For several nitrifiers the ability to switch between ´classical` nitrification, nitrifier-denitrification and aerobic denitrification was shown [*Wrage et al.*, 2001, *Whittaker et al.*, 2000, *Zart et al.*, 2000, *Zehr and Ward*, 2002]. The oxygen sensitivity is species-specific and also enzyme-specific; therefore the scatter of data might reflect the variety of involved species and enzymes [*Jiang and Bakken*, 2000; *Goreau et al.*, 1980; *Wetzel*, 1983; *Robertson et al.*, 1988; *Richardson*, 2000]. *Bauer* [2003] investigated NH_4^+ oxidizing bacteria in the eastern Gotland Basin, and found similar bacterial communities at different depths; their nitrification activities however depended on O_2 concentrations.

Therefore, the ability of nitrifiers to perform denitrifying processes and the lack of ´classical` denitrifying signals, a switch of N_2O producing processes by nitrifiers can be assumed. These findings are in agreement with the assumptions of *Rönner* [1983], who also assumed, that nitrification is the main N_2O production pathway in the Baltic Sea.

Alternatively, it is also possible to interpret the data from the hydrographical or temporal point of view. Figure 9 shows the same data set as shown in Fig. 8. This time the data set is grouped not according to the oxygen concentrations but to the affiliation to different basins. Station 286 was excluded due to its transitional character. At this station anoxic conditions in the deep waters were found similar to other stations in the western Gotland Basin, but H_2S concentrations were decreasing towards the bottom. This indicates beginning ventilation, however still too weak to lead to oxic conditions.

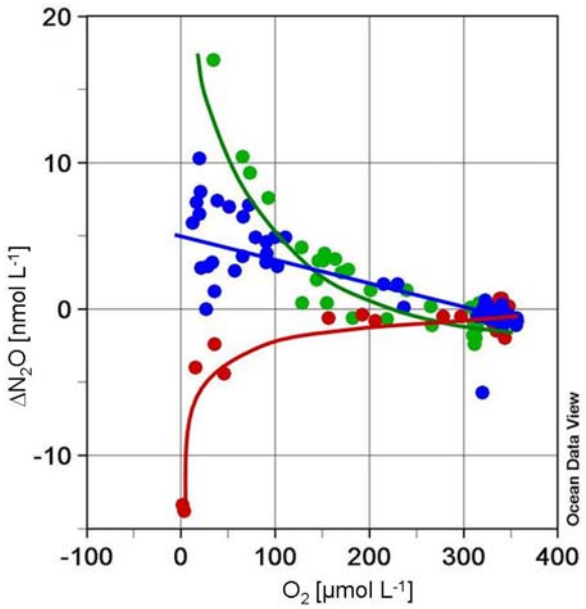


Fig. 9. Correlation between ΔN_2O and O_2 in the Baltic Sea. Correlations were calculated for the Bornholm Basin (station 140, 200, 213, 222, green coloured, $y = -6.83\ Ln(x) + 37.88$, $R^2 = 0.86$), the eastern Gotland Basin (station 259, 250, 260, 271, blue coloured, $y = -0.02\ x + 5.88$, $R^2 = 0.70$) and the western Gotland Basin (station 284, 240, 245, red coloured, $y = 2.25\ Ln(x) - 13.32$, $R^2 = 0.86$). Anoxic data and station 286 were excluded.

In the stratified basins such as the Bornholm Basin, and the eastern and western Gotland Basin correlations of ΔN_2O and O_2 were regionally different and not always linear (Fig. 5b-c, 6b-c, 7a, 9). Particularly in the Bornholm Basin, N_2O and oxygen as well as N_2O and nitrate showed significant non-linear relationships (Fig. 5b-c, 9). The Bornholm Basin, which was anoxic before the inflow [*Schmidt,* 2002]**,** was ventilated by North Sea Water in January 2003, months before the northern part of the eastern Gotland Basin was affected by the inflow [*Nausch,* 2003a, *Nausch et al.*, 2004]. In October 2003 the oxygen conditions were already switching back to suboxic conditions [*Nausch*, 2003c; *Wasmund*, 2003], visible by decreasing oxygen concentrations compared to the beginning of the year. Accordingly the duration of elevated oxygen concentration in the respective basins may contribute to the observed accumulation of N_2O. In the eastern Gotland Basin (Fig. 6b-c, 9) the anoxic conditions changed a few months after the Bornholm Basin: the Gotland Deep was ventilated by North Sea Water in May 2003 [*Nausch,* 2003b]. Thus, there was less time for N_2O accumulation. For various communities of NH_4^+ oxidizing bacteria different lag times after switching from anoxic to oxic incubations were shown and the production of N_2O might not have started directly after the ventilation by North Sea Water [*Bodelier et al.*, 1996]. In the western Gotland Basin (Fig. 7b-c, 9) no ventilation by North Sea Water had occurred by October 2003, therefore degradation of N_2O at

the oxic-anoxic interface was found. We suspect that the correlation between ΔN_2O and O_2 in the Bornholm Basin and the eastern Gotland Basin will become similar to those of the western Gotland Basin with time, when the conditions change to anoxic.

Summarizing, we assume a conversion of N_2O mainly by nitrifiers, depending on oxygen concentration and with significant spatial and temporal characteristics.

4.3 Estimated contribution of the North Sea Water inflow to the production of N_2O

The estimated N_2O content in the stratified basins showed distinctly higher values after the inflow of the North Sea Water than before. The N_2O concentration in the North Sea Water was assumed to be near equilibrium, so there was no significant advection of N_2O from the North Sea. Thus, the observed elevated N_2O concentrations in the Baltic Sea basins result from a stimulation of N_2O production by the inflow, most likely by advection of oxygen (see Table 1).

Although more than 1000 tons of N_2O were produced, it is questionable whether the North Sea Water inflow makes the Baltic Sea a source of atmospheric N_2O. Due to the strong salinity stratification, it can be assumed that the formed N_2O stays below the permanent halocline, and therefore it will not reach the atmosphere. Commonly N_2O budgets were modelled as a function of nitrification and denitrification. *Seitzinger and Kroeze* [1998] modelled the distribution of N_2O production, amongst others based on the input of nitrogen compounds into estuaries by rivers. However, estimations of global N_2O emissions do not or only to a small extent take into account the hydrographic aspects. The stratification of the water column probably lead to a reduced release of calculated amounts, and accordingly to an overestimation of N_2O emissions.

The assumption of remaining N_2O below the halocline leads to the question, whether and to what extent the nitrogen cycle might be influenced.

Based on the calculated N_2O content of the basins and the assumption of nitrification as the main production pathway N_2O production rates and nitrification rates were estimated (Table 2). These nitrification rates are in good agreement with previously published rates for the Baltic Sea [*Enoksson*, 1986; *Bauer* 2003]. *Bauer* [2003] calculated for the eastern Gotland Basin mean nitrification rates of 21.6 ± 11.1 nmol L^{-1} at 60 m depth, respectively 44.3 ±33.1 nmol L^{-1} at 100 m depth.

Table 2: Estimated nitrification rates in the Bornholm Basin and the eastern Gotland Basin, based on the assumption of 0.3 % N_2O release during nitrification [Seitzinger and Kroeze, 1998]

	Δm_{N2O} [tons]	d_{basin} [day]	Water volume [km^3]	N_2O production rate [nmol L^{-1} d^{-1}]	nitrification rate [nmol L^{-1} d^{-1}]
Bornholm Basin	220 ± 81	265	306	0.059 ± 0.023	19.62 ± 7.57
eastern Gotland Basin	920 ± 198	167	1195	0.105 ± 0.021	34.92 ± 6.87

These rates are comparably low [e.g. *Bianchi et al.*, 1999]; therefore the influence on the nitrogen cycle in the Baltic Sea might be small, too. N_2O might play a minor role as a reserve- or buffer-molecule during the change to anoxic conditions, preserving nitrifying processes in exchange for oxygen for a short while.

5. Conclusions

In January 2003 a major inflow of cold, highly saline and oxygen-rich North Sea Water was observed, terminating the ongoing stagnation period in parts of the central Baltic Sea.

- In agreement with previous studies, we found N_2O production mainly in oxic water masses below the Winter Water layer.
- We found no indication for advection of N_2O by North Sea Water; however, the environmental conditions for N_2O production were clearly changed due to the North Sea Water inflow.
- The inflow leads to a stimulation of N_2O production below the permanent halocline, but due to the halocline, the Baltic Sea is not a significant source of N_2O to the atmosphere.
- There was no indication for an accumulation of N_2O during denitrification. In oxic and suboxic water masses nitrification seems to be the main production pathway. The occurrence of nitrifier-denitrification and aerobic denitrification is possible, but needs further investigations.

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Chapter 6

Analysis of bacterial communities in the subtropical North Atlantic by 16S rRNA genes

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Abstract

The vertical composition of bacterial communities in the oceans is largely unknown, though bacteria play an important role in biogeochemical cycles, beside others in the production and consumption of greenhouse gases such as methane or nitrous oxide. Studies showed possible correlations between single picoplankton species and chemical compounds, i.e. between species related to the genus *Roseobacter* and the turnover of DMSP to DMS. Therefore, the hypothesis was that it might also be possible to find species which are associated with the distribution of nitrous oxide, an atmospheric trace gas with considerable relevance for the Earth's climate. We investigated the vertical distribution of bacteria in the western and eastern subtropical North Atlantic (Meteor 60-5 cruise), identified by 16S rRNA gene sequences, with the intention to find correlations between the community structure of bacteria and the distribution of nitrous oxide. In the subtropical North Atlantic N_2O is assumed to be mainly produced during nitrification. However, the functional genes encoding the enzymes for the conversion of N_2O during nitrification are unknown.

The yield of N_2O depends on environmental conditions and also on the species involved in conversion processes. *Proteobacteria* were found to be the predominant group in both basins. In the western basin the contribution of *Proteobacteria* increased significantly with depth, and also the composition changed with depth: α-*Proteobacteria* were predominant at shallower depth and γ-*Proteobacteria* increased in proportion in deeper water masses. Particularly *Acinetobacter* and *Alteromonas* seem to become more important with depth. In contrast, in the eastern basin *Proteobacteria* were also the predominant group of identified bacteria, but with minor proportions. Here no depth depending community structures were found. In both basins β- and δ-*Proteobacteria* hardly occurred. Particularly *Proteobacteria* are known to perform N_2O producing processes, however, we found no obviously predominant bacterial group related to the distribution of N_2O.

1. Introduction

Pelagic bacteria are a major component of biomass in oceanic oligotrophic systems [*Binder et al.*, 1996; *Cho and Azam*, 1990; *Carlson et al.*, 1996]. It is generally accepted that major bacterioplankton groups show a vertical stratification [*Lee and Fuhrman*, 1991; *Field et al.*, 1997], and community structures shift with changing environmental factors [*Bollmann and Laanbroek*, 2002]. Some studies showed that correlations between single picoplankton species and chemical compounds (e.g. species of *Roseobacter* and Dimethylsulfoniopropionate, DMSP) can be found [*Zubkov et al.*, 2001; *González et al.*, 2000]. Thus, there might exist a similar pattern for nitrous oxide (N_2O). N_2O is an atmospheric trace gas with a high climatic relevance. It is an intermediary and a by-product in several processes of the nitrogen cycle. The contribution of single processes of the nitrogen cycle to the global N_2O budget is discussed controversially [*Codispoti et al.*, 2001; *Popp et al.*, 2002; *Yamagishi et al.*, 2005], and the knowledge of biological processes and controlling factors of the production and conversion of N_2O in situ are still unsatisfactory.

Up to now nitrification and denitrification are commonly accepted to be the main N_2O producing processes in the nitrogen cycle [*Bonin et al.*, 2002]. In general, nitrification is an aerobic process in which organic or inorganic nitrogen compounds are oxidized to nitrate (NO_3^-) via hydroxylamine (NH_2OH) and nitrite (NO_2^-). In this process N_2O is considered as a by-product. It is assumed that N_2O is either produced by an incomplete conversion of NH_2OH or by oxidation of NO_2^- [*Ostrom et al.*, 2000; *Popp et al.*, 2002]. Denitrification is the reduction of NO_3^- to N_2, with N_2O as a regular intermediate product [*Naqvi et al.*, 2000]. The yield of N_2O strongly depends on abiotic factors like concentrations of oxygen and nitrate but is also species specific [*Brettar and Rheinheimer*, 1991; *Goreau et al.*, 1980; *Vollack and Zumft*, 2001; *Wetzel*, 1983; *Abou Seada and Ottow*, 1985].

1.1 Bacteria involved in the production of N_2O

1.1.1 Nitrifying bacteria

Nitrification can be conducted by both autotrophic and heterotrophic organisms. Typically two groups of microorganisms are involved in autotrophic nitrification: NH_4^+-oxidizing bacteria or primary nitrifiers and NO_2^--oxidizing bacteria or secondary nitrifiers [*Bock et al.*, 1986]. Both groups are affiliated with *Proteobacteria*. NH_4^+-oxidizing bacteria, obligatory lithotrophic, belong to the β- and γ-*Proteobacteria*, divided into the genera *Nitrosomonas*, *Nitrosococcus*, and *Nitrosospira* (including the genera *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobolus*) [*Bothe et al.*, 2000; *Krümmel and Harms*, 1982]. NO_2^--oxidizing bacteria belong to the α-*Proteobacteria* [*Schramm et al.*, 1998; *Woese et al.*, 1984a; *Woese et al.*, 1984b; *Woese et al.*, 1985], divided into the genera *Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira.* Among these, *Nitrobacter* and *Nitrospira* strains are capable of heterotrophic or mixotrophic growth, the rest being strict chemolithotrophs [*Bartosch et al.*, 1999]. The *Nitrospina* group was shown to be affiliated with the δ-subdivision of the *Proteobacteria* but did not share a specific relationship to each other or to other members of the δ-subdivision [*Abeliovich*, 2001]. Formerly autotrophic nitrifiers were classified as nitrifying bacteria in the family *Nitrobacteraceae* [*Buchanan*, 1917], but phylogenetically they are not closely related to each other [*Woese et al.*, 1984a; *Woese et al.*, 1984b; *Woese et al.*, 1985]. Both groups are widely distributed in marine environments [*Venter et al.*, 2004; *Gallagher et al.*, 2004; *Ward and O'Mullan*, 2002; *Morris et al.*, 2002].

Besides autotrophic nitrifiers also heterotrophic nitrifiers are known, which are phylogenetically more widespread and include a variety of species [*Mével and Prieur*, 2000]. They are unspecialized heterotrophic microorganisms for which their nitrogen oxidation property is not regarded as taxonomically significant [*Focht and Verstraete*, 1977].

1.1.2 Denitrifying bacteria

Denitrification reactions are widely distributed in various subclasses of the *Proteobacteria*, but also organisms of other eubacterial and archaeal genera are able to denitrify [*Chen et al.*, 2002; *Shapleigh*, 2001; *Cabello et al.*, 2004]. 16S rRNA and functional gene phylogenies are not congruent for denitrifiers, implying substantial horizontal gene transfer of the functional genes over time [*Zehr and Ward*, 2002; *Ward*, 2005]. However, this topic is discussed controversially [Petri and Imhoff, 2000].

Nitrifiers possess the ability to perform denitrifying processes as well, so called nitrifier-denitrification and aerobic denitrification [*Wrage et al.*, 2001]. Under aerobic conditions, both processes can be performed by either autotrophic NH_4^+ oxidizing bacteria or heterotrophic nitrifiers [*Wrage et al.*, 2001; *Whittaker et al.*, 2000; *Zart et al.*, 2000; *Zehr and Ward*, 2002]. Although the processes have identical educts and products as ´classical` denitrification, they differ by the structure of used enzymes and their genetics [*Moreno-Vivián et al.*, 1999; *Richardson and Watmough*, 1999; *Robertson and Kuenen*, 1988]. Many organisms are able to use different enzymes for similar reactions or to switch between these pathways depending on the environmental conditions [*Moir et al.*, 1995; *Richardson*, 2000; *Wrage et al.*, 2001].

Our studies focused on the vertical structure of bacterial communities in the subtropical North Atlantic, investigated by 16S rRNA genes. Aim was to obtain more information about the vertical distribution of bacteria, with the intention to find correlations between the structure of bacterial communities and the distribution of the greenhouse gas N_2O.

2. Methods

2.1 Sampling location

Samples were taken in the subtropical North Atlantic during March / April 2004 onboard the research vessel ´Meteor`. The cruise (M 60-5) started in Fort de France, Martinique (French Antilles) in the western part of the Atlantic and ended in Lisbon (Portugal).

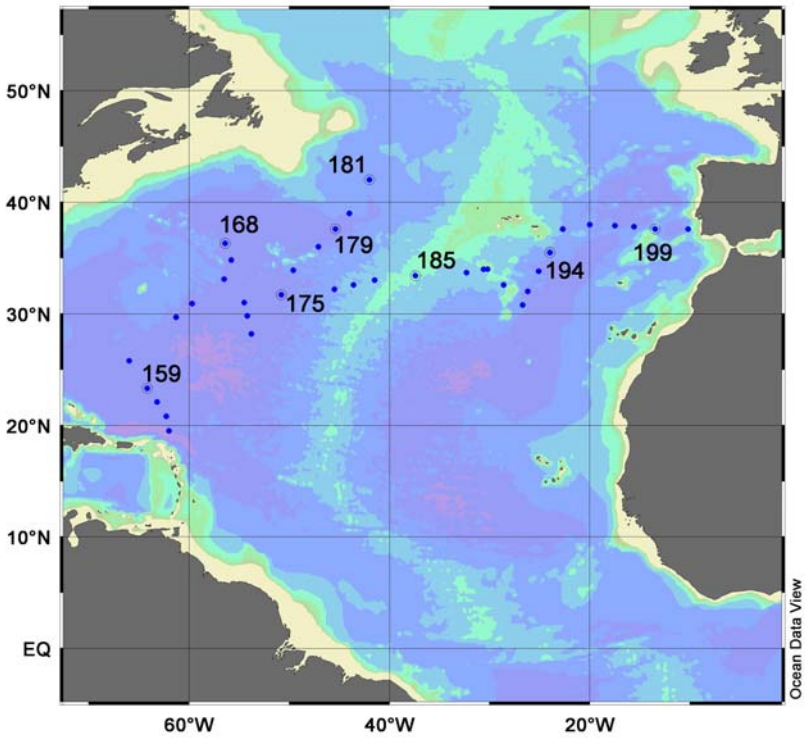

Fig. 1: Cruise track of M 60-5 in the subtropical North Atlantic; numbers mark sampled stations for molecular analysis and N_2O profiles, dots mark sampled stations only for N_2O profiles

2.2 N_2O measurements

Water samples for N_2O analysis were collected in triplicate at 37 stations from various depths, taken with a 24-Niskin-bottle rosette, equipped with a CTD-probe. N_2O was measured directly on board with a gaschromatograph, equipped with an ECD (Electron Capture Detector). The analytical method applied is described by Walter et al. [2005].

2.3 Sampling for molecular analysis

70 water samples were taken with a 24-Niskin-bottle rosette at 8 stations (see Fig. 1 for station numbers), at depths from the surface to the bottom. Depths were selected according to the shape of profiles of nitrous oxide and oxygen. A water volume of 1 L was filtered through 0.2 µm polycarbonate filter, which were immediately stored at - 80°C.

2.4 DNA-extraction

Until DNA-extraction filters were stored at -80°C, not exceeding five months after sampling. For extractions of total DNA one quarter of the filter was used. The extraction protocol followed standard methods [*Ward et al.*, 1993] using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Extracted nucleic acids were stored in DNA-free water at - 20°C.

2.5 PCR

16S rRNA genes were amplified using PCR by two different protocols for DGGE [*Muyzer et al.*, 1993] and cloning, with primer pairs specific for *Eubacteria* (see Tab. 1 and Tab. 2). The PCR for DGGE was performed as a touchdown-PCR; a GC-clamp was attached to the used primer.

For these reactions 1 µL of each primer (10 pmol L^{-1}, see table 1) and 1 µL DNA-extract were used, and PCR was performed using Ready-To-Go™ PCR Beads (Amersham Pharmacia Biotech). The amplification results were checked by agarose gels (1% agarose, 1 x TBE). The PCR products were purified with a High Pure PCR purification Kit (Roche, Mannheim, Germany), and used directly for following DGGE or cloning experiments.

Table 1: PCR protocol for DGGE, * temperature was reduced for 1 °C at each cycle

cycles	**annealing**		**elongation**		**denaturation**	
1					94 °C	120 s
15	*65 – 50 °C	40 s	72 °C	40 s	94 °C	30 s
40	50 °C	40 s	72 °C	40 s	94 °C	30 s
1	42 °C	60 s	72 °C	300 s		
99	4 °C					
primer: 5´342 GC	([CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GC] CTA CGG GAG GCA GCA G)					
3´534	(ATT ACC GCG GCT GCT GG)					

Table 2: PCR protocol for cloning

cycles	annealing		elongation		denaturation	
1					94 °C	120 s
30	50 °C	40 s	72 °C	40 s	94 °C	30 s
1	42 °C	60 s	72 °C	300 s		
99	4 °C					
primer: 5´27F (GAG TTT GAT CCT GGC TCA G)						
3´1387 (CGG GCG GTG TGT ACA AGG)						

2.6 DG-DGGE

Bacterial diversity was qualitatively investigated by a double gradient modification DGGE (DG-DGGE) [*Petri and Imhoff*, 2001] at 8 stations (see Fig.1, stations marked with numbers). DG-DGGE was performed using a CBS 2001 Scientific System (Del Mar, Calif.). The gels consisted of a denaturant gradient (50-80%), superimposed with an acrylamide gradient (6 – 8 %). For electrophoresis of 25 µL PCR-product a 0.5 TAE buffer was used at a constant voltage of 80 V and 60 °C for 14 h. Afterwards gels were stained with an ethidium bromide solution (0.5 mg L^{-1}) for 30 min, washed with MilliQ water for 30 min, and documented by a Kodak 1D Image analysis system .

2.7 Cloning and sequencing

For cloning and sequencing experiments two representative stations with four, respectively three depths were selected: station 175 in the western basin of the subtropical North Atlantic (31.747 °N, -50.752 °E) and station 194 in the eastern basin (35.525 °N, -23.982 °E). The depths were selected by measured N_2O profiles. For both stations the depth of the N_2O maximum was chosen, additionally depths above and below. Cloning experiments were performed with a TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's manual. Clones were cultivated (for 24 h at 37 °C) on a LB medium containing 50 µg mL^{-1} canamycin, and afterwards 96 colonies of each cloning experiment were picked for sequencing. Sequencing was performed commercially (AGOWA, Berlin).

2.8 Sequence analysis and phylogeny

Sequences were manually refined and edited with the program Seqman (DNAstar, Lasergene, Madison, USA), and compared to the Ribosomal Database Project, release 0.9, (http://rdp.cme.msu.edu/classifier/classifier.jsp), on the basis of the Bergey's Manual of Systematic Bacteriology, with a confidence threshold of 80 %. Phylogenetic analyses were made with the ARB software [*Strunk et al.*, 1999].

3. Results

3.1 Physical and chemical parameters

The physical and chemical parameters (at the representative stations 175 in the western basin and station 194 in the eastern basin) at depths sampled for sequence analyses are shown in Table 1.

Table 3: Physical and chemical conditions

	station 175				station 194		
	400 m	800 m	1200 m	4000 m	400 m	1000 m	1500 m
temperature (CTD)	16.63	9.28	5.88	2.31	13.71	9.07	5.76
salinity (CTD)	36.33	35.29	35.20	34.95	35.85	35.59	35.25
oxygen [µmol L^{-1}]	199	160	225	263	213	188	239
oxygen sat. [%]	70.3	55.7	72.5	77.1	82.0	65.4	76.7
nitrate [µmol L^{-1}]	7.36	22.2	19.8	20.3	9.8	18.9	18.2
nitrite [µmol L^{-1}]	0.01	0.0	0.0	0.0	0.0	0.0	0.0
phosphate [µmol L^{-1}]	0.41	1.4	1.3	1.4	0.7	1.2	1.2
silicate [µmol L^{-1}]	2.52	12.1	12.9	35.9	3.7	11.3	12.3
pot. density [kg m^{-3}]	26.6	27.3	27.7	27.9	26.9	27.6	27.8

N_2O distributions and profiles in the subtropical North Atlantic showed strong variations with water depth (Fig. 2). The profiles generally had one distinct maximum. In the surface layer concentrations were uniform, increasing below the thermocline up to a maximum and decreasing down to approximately 2000 m. Below 2000 m N_2O concentrations were nearly constant with depth in both basins.

a) b)

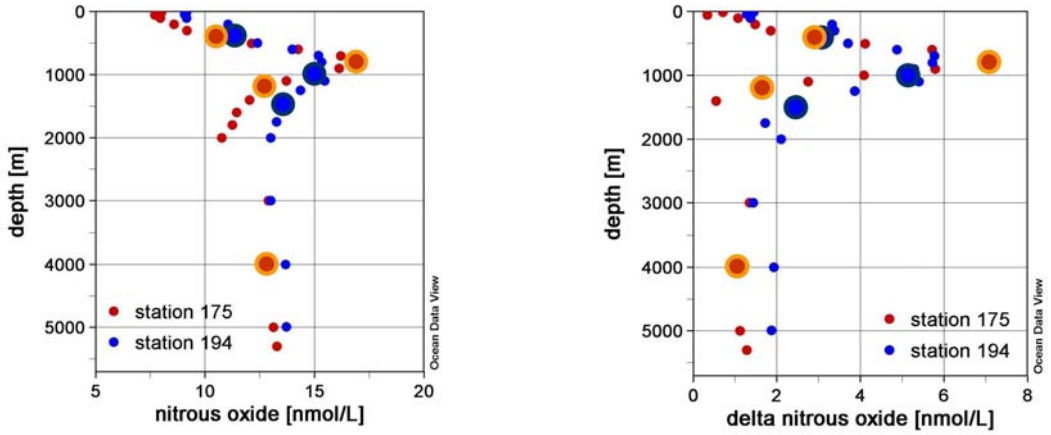

Fig. 2: Profiles of a) N_2O and b) ΔN_2O at station 175 and 194; depth selected for sequencing are marked

Surface concentrations were 8.7 ± 0.7 nmol L^{-1}, maximum values were found at depths between 600 to 1000 m; values ranged from 14.0 in the eastern basin (station 195) to 21.3 nmol L^{-1} in the western basin (station 156). Below 2000 m, concentrations were nearly constant at 13.1 ± 0.9 nmol L^{-1}. Profiles in the western subtropical North Atlantic showed distinct maxima, while in the eastern basin no clear maximum was expressed. From the western to the eastern basin maximum concentrations decreased slightly from 17.7 ± 1.4 nmol L^{-1} to 15.1 ± 0.7 nmol L^{-1}. East of the Midatlantic Ridge maxima were not clearly expressed and were broader. Additionally, maximum ΔN_2O values were lower in the eastern (5.5 ± 0.6 nmol L^{-1}) than in the western basin (7.9 ± 1.3 nmol L^{-1}).

3.2 DGGE

Figure 3 shows the gels of two DG-DGGE from station 175 in the western and station 194 in the eastern Basin of the subtropical North Atlantic (see Fig. 1). On both gels clearly different band patterns in different depths were found, although most bands were common to all samples. The band patterns of both stations differ in the composition of bands and the strength of those (at depths selected for sequencing some bands differing between the depths are exemplarily marked by red and blue circles). Therefore, a qualitatively different bacterial community in different depths of the water column can be assumed.

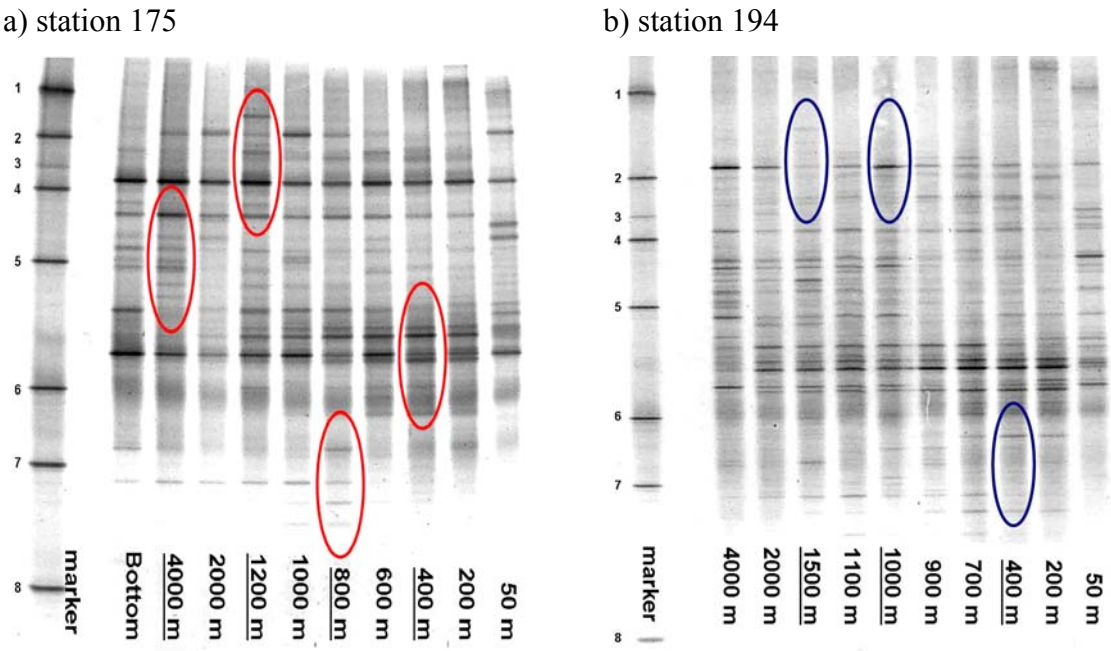

Fig. 3: DG-DGGE of eubacterial amplified 16S rDNA fragments covering a depth profile of the water column of station 175 (a) and station 194 (b); the left column shows an internal marker. The depth selected for cloning and sequencing experiments were underlined, differences between them were exemplarily marked with red and blue circles, note the position of marker and bands at each station.

3.3 Sequence analysis and phylogeny

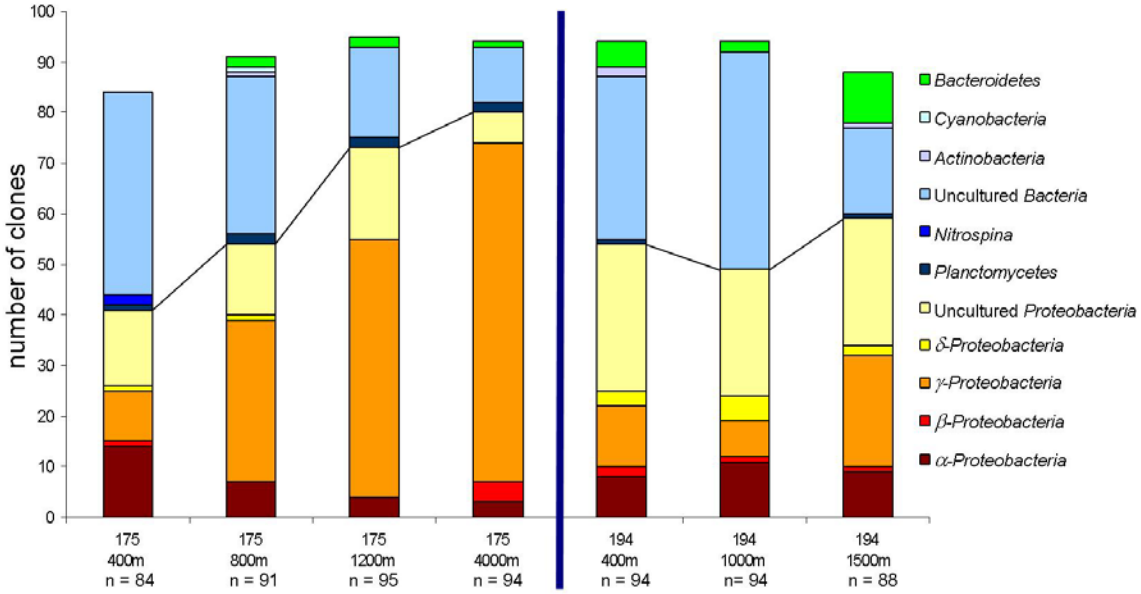

Fig. 4: Total number of clones of bacterial groups, at station 175 and 194 at different depths, identified by the Ribosomal Database Project using a confidence threshold of 80 % http://rdp.cme.msu.edu/index.jsp, lines between the bars separate *Proteobacteria* from other identified groups of bacteria, n gives the total number of sequenced clones

In total 640 clones were sequenced (mean length of sequences was 703 bases), and first analysis of sequences using the Ribosomal Database Project (Fig. 4) supported the results of the DGGE-gels that the bacterial communities differed with depths.

All clones of the clone libraries were strained into six major lineages of the domain bacteria: *Proteobacteria*, *Planctomycetes*, *Nitrospira*, *Actinobacteria*, *Cyanobacteria,* and *Bacteroidetes*. A large part of clones were most closely related to uncultured, environmental sequences. At station 175, the contribution of clones related to uncultured sequences decreased continuously with depth, whereas at station 194 those clones were mainly found at 1000 m.

Sequences affiliated to the *Proteobacteria* were the predominant group in both basins, followed by those related to uncultured bacteria. The composition and contribution of *Proteobacteria* was different depending on location and depth. In both basins sequences of the β- and δ-subdivision of *Proteobacteria* were hardly represented, whereas sequences related to α- and γ-*Proteobacteria* contribute to a major part to the total number of clones.

In the western basin the contribution of *Proteobacteria*-sequences increased significantly with depth (48.9 – 85.2 %), with changing composition: sequences related to α-

Proteobacteria were predominant at shallower depth, whereas sequences related to γ-*Proteobacteria* increased in proportion in deeper water masses. In contrast, at station 194 in the eastern basin sequences of α- and γ-*Proteobacteria* were also the predominant group, but showed no clear depth depending distribution. The number of clones affiliated to α-*Proteobacteria* was higher in the eastern basin than in the western, conversely to the number of clones affiliated to γ-*Proteobacteria*.

This is also obvious in the correlation of clone numbers of α- and γ-*Proteobacteria* with the potential density (Fig. 5). No correlations were found in the eastern basin at station 194. In contrast, in the western basin at station 175 the number of clones related to the α-*Proteobacteria* were found negatively correlated with the potential density, whereas those of γ-*Proteobacteria* were positively correlated.

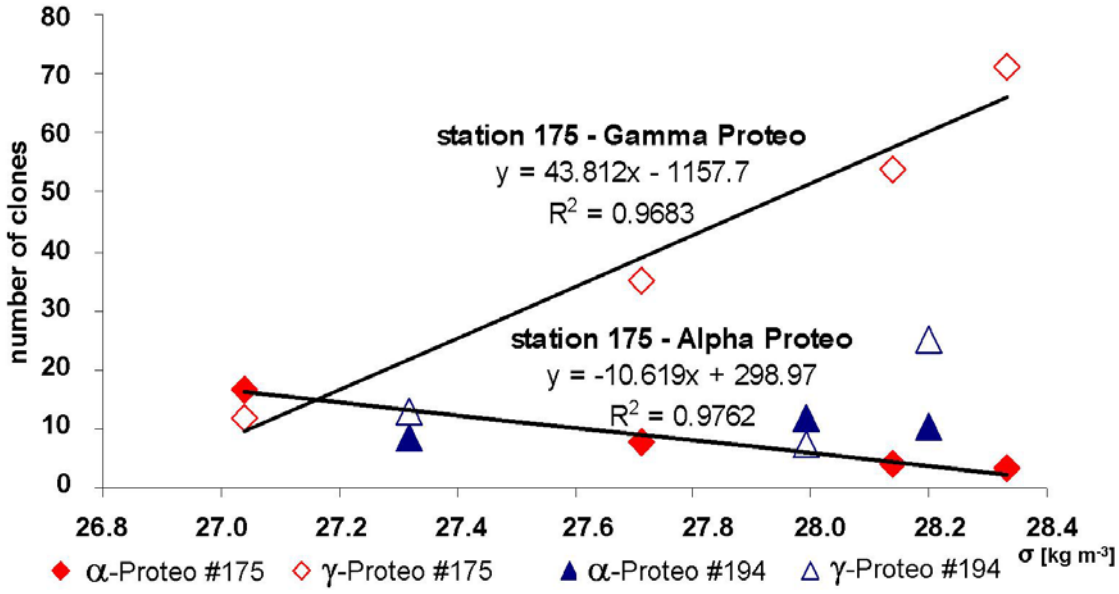

Fig. 5: Correlation between the clone number of α- and γ-*Proteobacteria* (given as a percentage of the number of clones in total) and the potential density [kg m^{-3}] at station 175 and station 194

Beside the increasing contribution of *Proteobacteria*-sequences and the differences between the contributions of sequences related to the α- and γ-*Proteobacteria* with depth, the composition of these subdivisions also changed depending on depth (see Fig. 6). This is obvious especially within the γ-subdivision.

At station 194, a depth depending contribution of the γ-subdivision of the *Proteobacteria* was not found, but the number of genera increased more than threefold (3 up to 10) from 400 m to 1500 m. In 1500 m particularly the clone numbers of members of the family *Alteromonadaceae* (*Alteromonas*, *Pseudoalteromonas*) increased.

At station 175 the total number of clones of the genera *Alteromonas* and *Acinetobacter* increased clearly with depth, at 4000 m a contribution of 68 % to the number of sequences affiliated to the *Proteobacteria* was found.

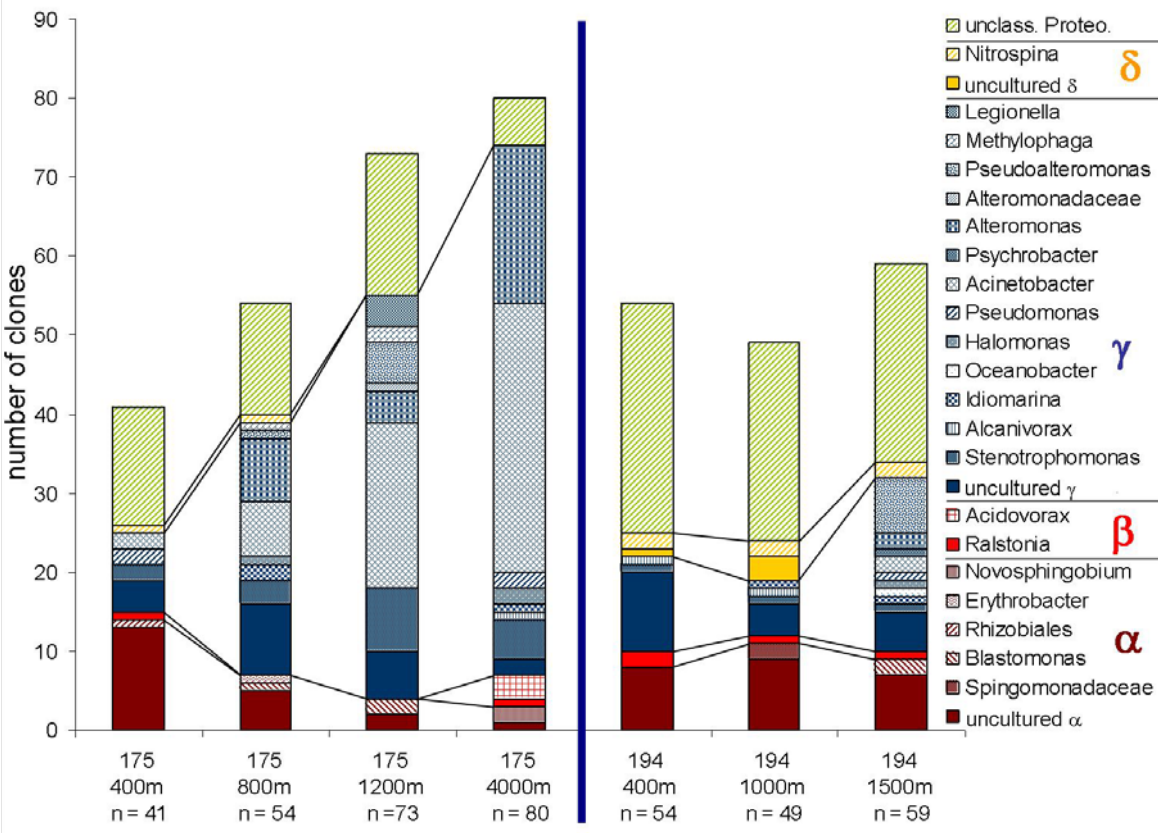

Fig. 6: Number of clones of the *Proteobacteria* groups, at different depths of station 175 and 194, n gives the number of identified clones by the Ribosomal data base using a confidence threshold of 80 % http://rdp.cme.msu.edu/index.jsp, lines between the bars separate the subdivisions of *Proteobacteria* from each other , the order of genera given in the legend correspond to the order of genera in the bars (top down)

A more detailed picture of phylogenetic relationships is given by analysis with the program ARB [*Strunk et al.*, 1999]**.** In general, the classification of sequences by ARB and RDP coincide. Most of the groups were found at both stations, with some exceptions. Seven sequences were affiliated with the group *Acidobacteria* (Fig. 10, highlighted in orange). These sequences were only found at station 194 in the eastern basin. Sequences affiliated to the groups *Piscirckettsiaceae*, *Spirochaetes*, *Verrucomicrobiae*, *Planctomycetes*, *Nitrospira,* and to one cluster related to uncultured sequences (Fig. 8, 10) , were only found at station 175 in the western basin of the subtropical North Atlantic. In the α- and δ-subdivision of *Proteobacteria* (Fig. 7, 9) we found also cluster associated with only one basin.

A major number of sequences are affiliated to uncultured, environmental sequences, particularly in the α- and the δ-subdivision of the *Proteobacteria.* The closest relatives of most sequences related to the α-*Proteobacteria* (Fig. 7, group of uncultured bacteria with 151 of our sequences) are *Pelagibacter ubique* (1972; AF510191 and 1974; AF510192), a very small, heterotrophic bacterium, which is present throughout the oceans [*Giovannoni et al.*, 2005; *Morris et al.*, 2002]. A more detailed picture about the phylogenetic classification is given by trees exported from ARB (Fig. 7-10)

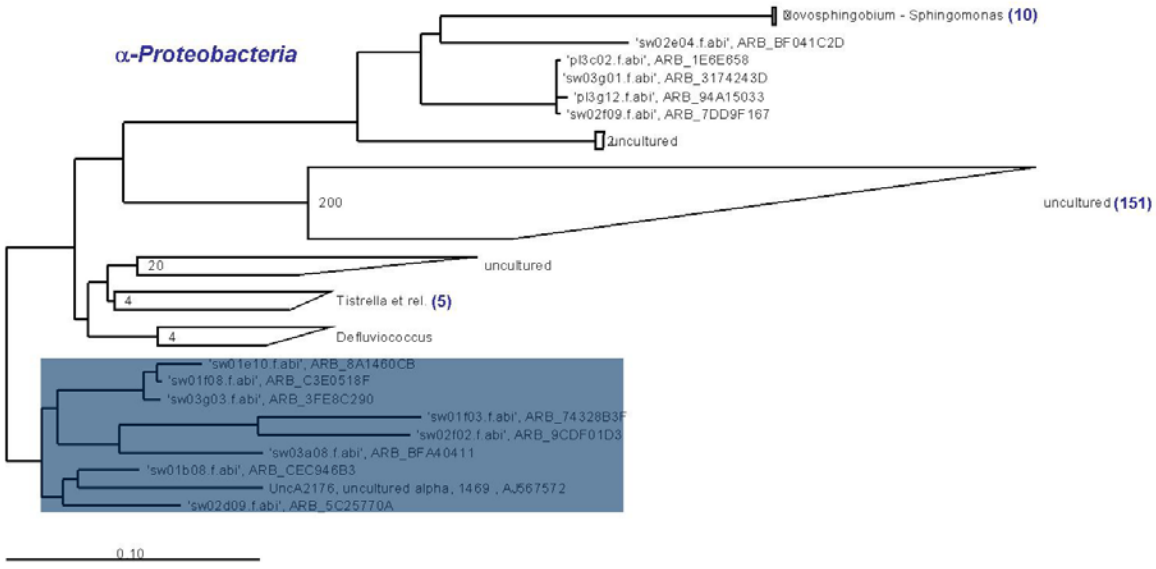

Fig. 7: Phylogenetic tree of 16S rDNA sequences affiliated to α-*Proteobacteria* exported from ARB (Maximum Parsimony); signatures of sequences obtained during this study start with the letters "sw" and "pl"; the number of sequences in each group is given in parentheses; groups of sequences highlighted in blue were found at station 175; uncoloured groups contain sequences of both stations

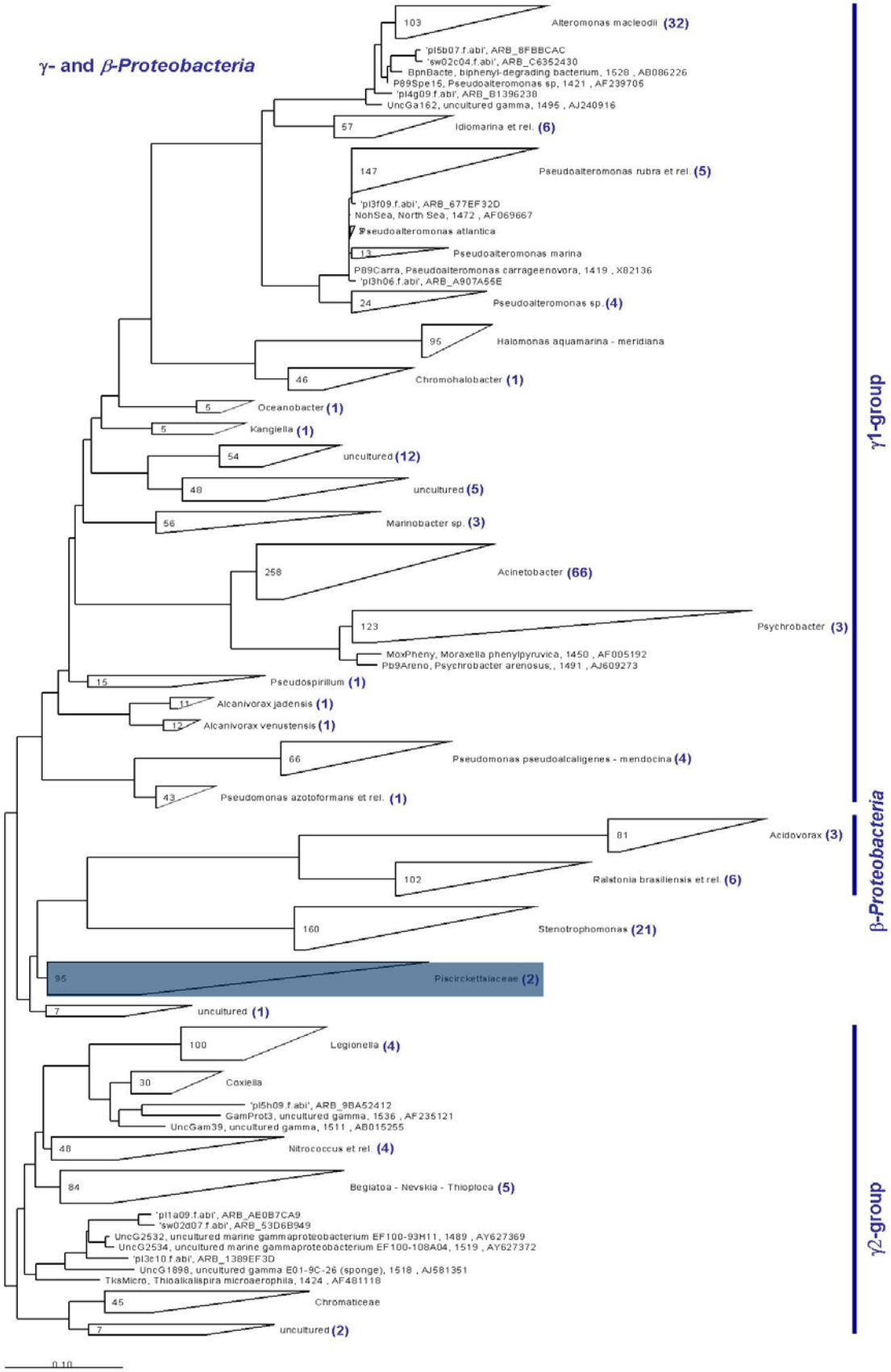

Fig. 8: Phylogenetic tree of 16S rDNA sequences affiliated to γ- and β-*Proteobacteria* exported from ARB (Maximum Parsimony); signatures of sequences obtained during this study start with the letters "sw" and "pl"; the number of sequences in each group is given in parentheses; groups of sequences highlighted in blue were found at station 175; uncoloured groups contain sequences of both stations

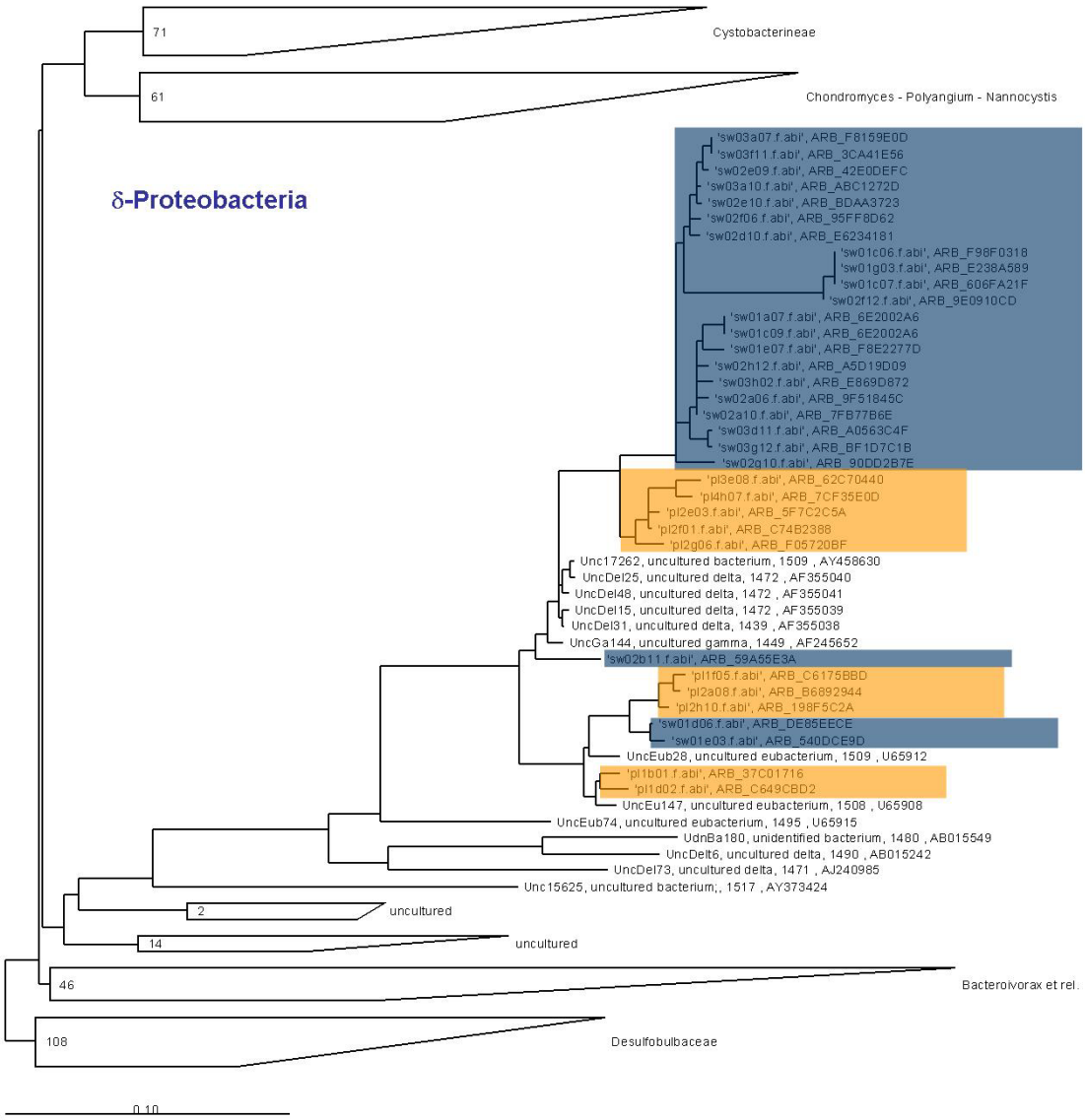

Fig. 9: Phylogenetic tree of 16S rDNA sequences affiliated to δ-*Proteobacteria* exported from ARB (Maximum Parsimony); signatures of sequences obtained during this study start with the letters "sw" and "pl"; groups of sequences highlighted in blue were found at station 175, groups of sequences highlighted in orange were found at station 194; uncoloured groups contain sequences of both stations

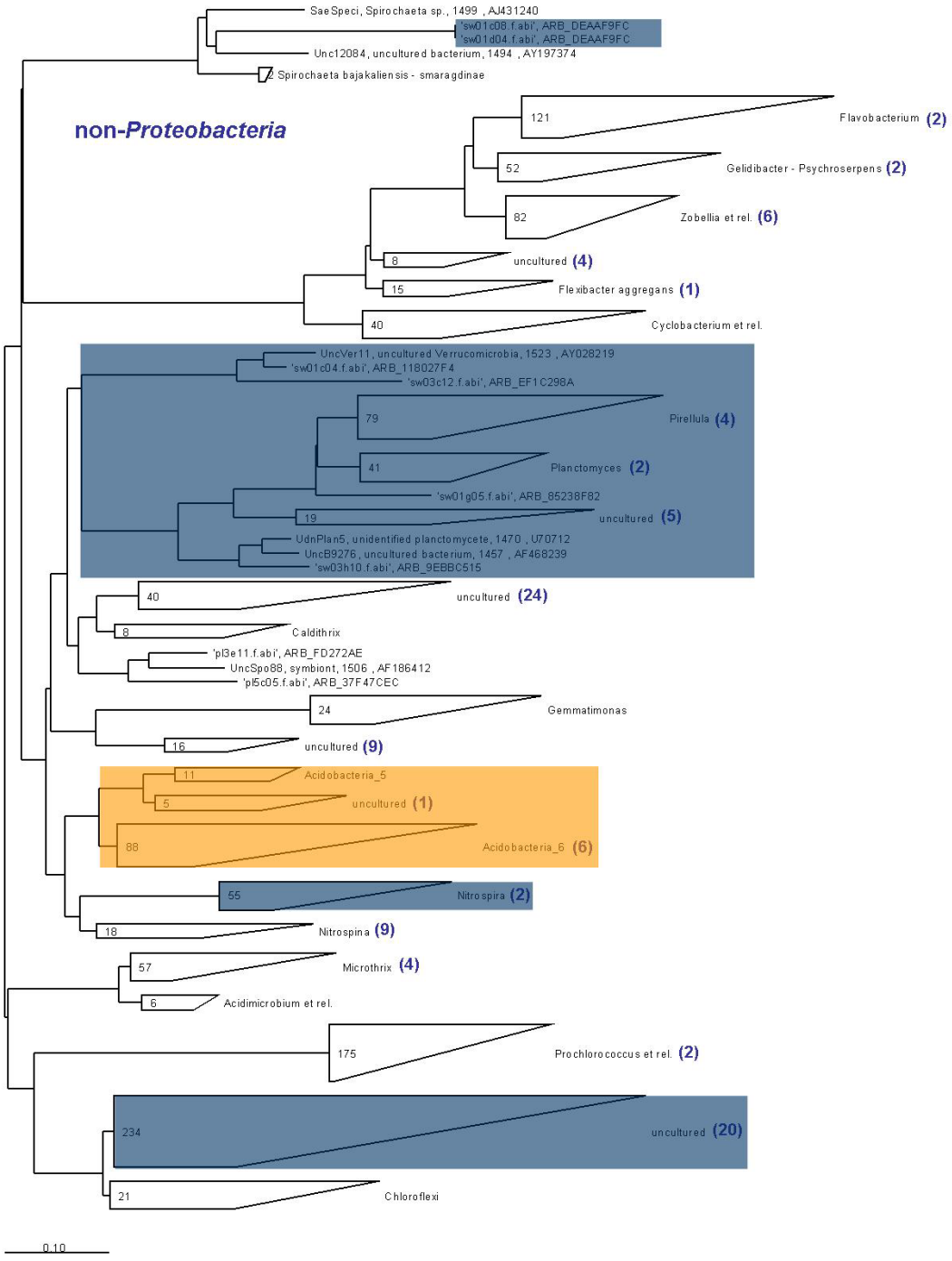

Fig. 10: Phylogenetic tree of 16S rDNA sequences affiliated to non-*Proteobacteria* exported from ARB (Maximum Parsimony); signatures of sequences obtained during this study start with the letters "sw" and "pl"; the number of sequences in each group is given in parentheses; groups of sequences highlighted in blue were found at station 175; groups of sequences highlighted in orange were found at station 194; uncoloured groups contain sequences of both stations

4. Discussion

16S rRNA genes have been used in several studies to analyze bacterial communities in the oceans [*Bano and Hollibaugh*, 2002; *Braker et al.*, 2001; *Corre et al.*, 2001; *Fuhrman and Davis*, 1997; *Gallagher et al.*, 2004; *Kato et al.*, 1996; *López-García et al.*, 2003; *Mullins et al.*, 1995; *Schmidt et al.*, 1991; *Ward and O'Mullan*, 2002]. However, most work was done on specific groups of bacteria or restricted to single depths, mainly to the surface layer or the upper water column and the sediments. Only few studies provide data of the oceanic water column from the surface to the deep sea, using "universal" primers to investigate the bacteria community in total [*Gallagher et al.*, 2004; *Tholosan et al.*, 1999; *Lee and Fuhrman*, 1991; *Maruyama et al.*, 1997]. The prokaryotes inhabiting the water column at depth greater than 1000 m are still largely unknown [*Gallagher et al.*, 2004].

The structure and vertical distribution of bacterial communities presented here generally coincide with previous findings. Sequences were affiliated only with a few major lineages of the domain *Bacteria*, dominated by members of the group *Proteobacteria* [*Bano and Hollibaugh*, 2002; *López-García et al.*, 2001; *Morris et al.*, 2002]. The dominance of subdivisions of the *Proteobacteria* depended on depths: sequences related to the α-subdivision were more present in the surface layer [*Bano and Hollibaugh*, 2002], conversely to members of the γ-subdivision, which were predominant in the deep sea [*López-García et al.*, 2001]. The vertical stratification leads to the interpretation that these groups are specialized to exploit vertical patterns in physical, chemical, and biological factors. The specialization to life at certain depths was shown in the ocean particularly for bacteria living at the surface and in the deep sea. Barotolerant and barophilic deep-sea bacterial strains belong to the genera of the γ-subdivision of *Proteobacteria*, namely *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and one new group [*Kato et al.*, 1996; *DeLong et al.*, 1997]. We found only small numbers of sequences belonging to these genera; the majority of sequences related to the γ-*Proteobacteria* in the deep sea belonged to the genera *Alteromonas* and *Acinetobacter*, with the closest affiliate to *Alteromonas macleodii* and *Acinetobacter junii*. In contrast, *Maruyama et al.* [1997] investigated the bacterial community at different depth in the Japan Trench, and found *Acinetobacter* as a main species in surface communities. However, *DeLong et al.* [1997] assume that the ability for growth under high pressure conditions might be fairly widely

distributed among the γ-*Proteobacteria*, and *Imada et al.* [2003] described barophilic strains of Acinetobacter in sediment samples collected from Sagami Bay and Enshu-Nada. Beside this, the concentration and composition of available nutrients differ clearly between surface and deep waters, whereas easily decomposable nutrients are depleted in deep waters [*Handa and Tominaga*, 1969; *Handa et al.*, 1992]. The genera *Acinetobacter* and *Alteromonas* are known to be able to use an extremely wide variety of organic compounds [*Towner*, 2001; *Mikahailov et al.*, 2002]. Therefore, the large number of sequences affiliated with *Acinetobacter* and *Alteromonas* might be not uncommon.

Differences between the stations might likely be explained by hydrographical aspects (Fig. 11) Phylogenetic analyses show that the majority of bacterial small subunit rRNA (SSU rRNA) gene lineages retrieved from near the coast of Oregon in the Pacific Ocean are related to those recovered from open ocean regions of both the Pacific and Atlantic Oceans, the Mediterranean Sea, and previously investigated coastal regions [*Rappé et al.,* 2000]. With some exceptions, at both stations the same major lineages of Bacteria were found (Fig. 4, 6, 7-10), but with clearly different contributions of different groups to the bacterial community. Particularly at depths of 1200 m in the eastern and 1500 m in the western basin the chemical and physical conditions are not such different to explain the variety of bacterial community solely (Table 3, Fig. 11). Therefore, the distribution and contribution of water masses seem to have a strong influence on the bacterial community in the subtropical North Atlantic.

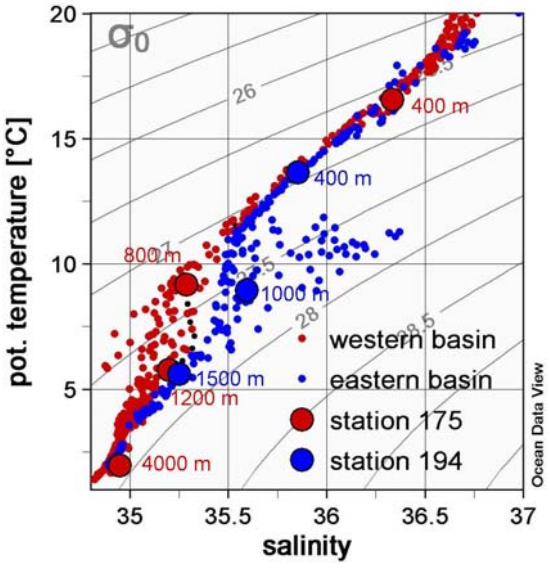

Fig. 11: T-S-diagram of the M 60-5 cruise, colour-divided into western and eastern basin , depths selected for sequencing of bacterial 16S rDNA clones are marked

Aim of this study was to investigate the composition of bacterial communities in the subtropical North Atlantic, with the intention to find correlations between the structure of bacterial communities and the distribution of the greenhouse gas N_2O. In the North Atlantic N_2O is assumed to be produced mainly by nitrification [see Nitrous oxide in the North Atlantic Ocean, chapter 2 of this thesis], therefore we expected sequences related to well-known aerobic and autotrophic nitrifying communities, such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira, and Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira.* Surprisingly, only a few of our sequences were affiliated to these genera, particularly to the genera *Nitrospina* and *Nitrospira*. This might be a methodical artefact, i.e. caused by PCR bias or due to different rRNA gene numbers [*v. Wintzingerode et al.*, 1997]. *Krawiec and Riley* [1990] showed a trend between fewer copies of rRNA gene regions (*rrn* operons) and slower growth of organisms, which is known for nitrifiers [*Ward*, 2005]. Both would lead to an underestimation of the contribution of autotrophic nitrifiers, respectively an overestimation of bacteria, whose sequences amplify with more efficiency to the primers or reveal more copies of the rRNA gene. However, autotrophic nitrifiers are very slow growing organisms with generation times on the order of a day, and they occur in relatively low abundances in natural environments [*Ward*, 2005]. So, despite methodical artefacts, there is also the possibility that the small number of sequences affiliated with nitrifiers might approximately mirror the contribution of autotrophic nitrifiers to the bacterial community.

Beside autotrophic nitrification, heterotrophic nitrification is also an opportunity for N_2O production. Commonly the relevance of heterotrophic nitrifiers within the nitrogen cycle was considered to be low, due to their lower nitrification rates in comparison to autotrophic nitrifiers [*Knowles*, 1985; *Jetten et al.*, 1997]. However, in recent years increased attention has been paid to heterotrophic nitrifiers because many of them are found to denitrify their nitrification products simultaneously [*Robertson et al.*, 1988; *Nishio et al.*, 1998]. This might lead to a significant underestimation of their nitrification ability and the release of N_2O by heterotrophic nitrifiers. *Papen et al.* [1989] found heterotrophic nitrifiers producing much more N_2O per cell than autotrophic nitrifiers. Thus, since heterotrophic nitrifiers are more widespread and include a variety of species [*Mével and Prieur*, 2000], they may contribute significantly to the global N_2O budget [*Jetten et al.*, 1997]. Heterotrophic nitrifiers which are able to produce N_2O are reported i.e. for strains of the γ-proteobacterial genus *Stenotrophomonas* [*Finkmann et al.*, 2000]. Due to the

relation of a major part of sequences to uncultured clones, we have little or no information on the physiology of these groups. Additionally, suggestions of shared metabolic characteristics between neighbouring groups are sometimes dangerous due to the immense diversity and capability of bacteria for horizontal gene transfer and rapid mutation [*Ward*, 2005].

Further investigations are necessary, to obtain a more detailed picture about the activities of bacterial communities. Fluorescence-in-situ-hybridization experiments might be helpful to prove the activity of involved genes, or antibody-based proof of enzymes and measurements of their activity. Beside the focus on members of the domain *Bacteria*, also *Archaea* should be taken into account. *Archaea* are also widely distributed in the oceans [*DeLong*, 1992; *Fuhrman and Davis*, 1997], and also able to perform different reductive pathways of the N-cycle, including both assimilatory processes, such as nitrate assimilation and N-2 fixation, and dissimilatory reactions, such as nitrate respiration and denitrification [*Cabello et al.,* 2004]. Archaeal denitrifiers generally stop denitrification activities at the production of N_2O [*Ward*, 2005], therefore *Archaea* might contribute, maybe even under oxic conditions, to the observed distribution of N_2O.

5. Summary

- The structure of the bacterial communities in the North Atlantic Ocean clearly depends on depths and sampling sites.
- *Proteobacteria* were predominant in the western as well as in the eastern basin, but with clearly different contribution of subdivisions and genera.
- Due to limited data an obvious relationship between N_2O distribution and single bacteria groups could not be proven.
- Heterotrophic nitrifiers or other N_2O producer might be important due to the low number of sequences affiliated with known autotrophic nitrifiers.

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Chapter 7

Influence of $HgCl_2$–poisoning and temperature on N_2O-concentrations during storage

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Abstract

Sometimes it is necessary to store samples until measurements. Commonly samples for N_2O determination are poisoned with saturated aqueous $HgCl_2$ solution to prevent biological activity until measurements. To investigate the effect of storage on N_2O concentrations at different conditions, we started an experiment over more than ten months. Samples were poisoned in three different ways, and stored at two different temperatures. With one exception, no significant change in N_2O concentrations was found after 10 months either in different poisoned treatments or in treatments stored at different temperatures. Only not poisoned samples, stored at room temperature, showed a distinct decrease in N_2O concentrations after 33 days to a concentration of approximately zero. Thus, it is possible to store samples for N_2O measurements for at least ten months after sampling.

1. Introduction

Due to temporal and spatial limitations during research cruises, it is not always possible to measure the obtained samples onboard. In this case samples need to be stored until the measurements can be conducted. However, in order to use stored samples it is necessary to know, how long samples can be stored without significant changes of parameters of interest e.g. dissolved N_2O. Commonly, to prevent biological activity until measurements, aqueous samples are poisoned with a saturated aqueous $HgCl_2$ solution, which acts as a strong cell and protoplasm toxin [*Glud et al.*, 1995; *Hemond and Duran*, 1989; *Jensen et al.*, 1984; *Kaplan et al.*, 1978; *McElroy et al.*, 1978; *Naqvi et al.*, 1994; *Oudot et al.*, 2002]. However, the use of saturated solutions seems to be not necessary because non-saturated aqueous $HgCl_2$ solutions are already highly toxic for aquatic organisms. For example the EC_5 (effective concentration) for organisms such as *Pseudomonas putida* is 0.01 mg L^{-1} (16h) [Safety data sheets of $HgCl_2$, http://uk.chemdat.info/mda/uk/, product number 104419]. Additionally low concentrated aqueous $HgCl_2$ solution may facilitate the handling of the solution during ship expeditions.
To investigate the effect of storage time on dissolved N_2O we collected surface water samples from the Kiel Fjord (Baltic Sea), and measured N_2O concentrations after different time periods during more than ten months. We applied different treatments of the samples regarding the degree of poisoning and storage temperatures.

2. Methods

We took surface water samples from the pier in front of the "Leibniz-Institut für Meereswissenschaften" (Kiel) at the 4th of September 2003. The analytical method applied is a modification of the method described by *Bange et al.* [2001]. 162 bubble free samples were taken, sealed directly with butyl rubber stoppers, and crimped with aluminium caps. Directly after sampling one third of the samples was treated with 300 µL per vial of a low concentrated mercury chloride (Merck, for analysis) solution of 40 mg L^{-1}, one third with 300 µL saturated mercury chloride solution (6 g L^{-1}) and the last third of the samples were not poisoned. At the first day (t = 0) we measured six samples of each treatment. Then the remaining samples were divided into storage at room temperature (in the lab, temperatures were between 17 – 25 °C) and storage at 4 °C in the fridge. All samples were stored in a paper board; therefore they were not exposed to light. For each treatment (poison, temperature) we measured six samples.

N_2O water concentrations (C_w) were calculated as follows:

$$C_w \left[\text{nmol L}^{-1}\right] = \left(\beta \text{ x P } V_{wp} + \frac{\text{x P}}{\text{R T}} V_{hs} \right) / V_{wp}$$

where β stands for the Bunsen solubility in nmol L^{-1} atm^{-1} [*Weiss and Price*, 1980], x is the dry gas mole fraction of N_2O in the headspace in ppb, P is the atmospheric pressure in atm (set to 1 atm), V_{wp} and V_{hs} stand for the volumes of the water (14 mL) and headspace phases (10 mL), respectively. R is the gas constant (8.2054 10^{-2} L atm mol^{-1} K^{-1}) and T is the temperature during equilibration.

For calibration we used standard gas mixtures with 311.8 ± 0.2 ppb and 346.5 ± 0.2 ppb N_2O in synthetic air (DEUSTE Steininger GmbH, Mühlhausen, Germany). The standard mixtures have been calibrated against the NOAA (National Oceanic and Atmospheric Administration, Boulder, Co.) standard scale in the laboratories of the Air Chemistry Division of the Max Planck Institute for Chemistry, Mainz, Germany). The overall mean analytical precision was ± 0.66 %.

The effectiveness of low concentrated $HgCl_2$ was additionally tested optically. 300 µL were added to a surface sea water probe with living flagellates, recognizable by agile movements.

3. Results and discussion

N_2O concentrations of different treatments are shown in Figure 1.

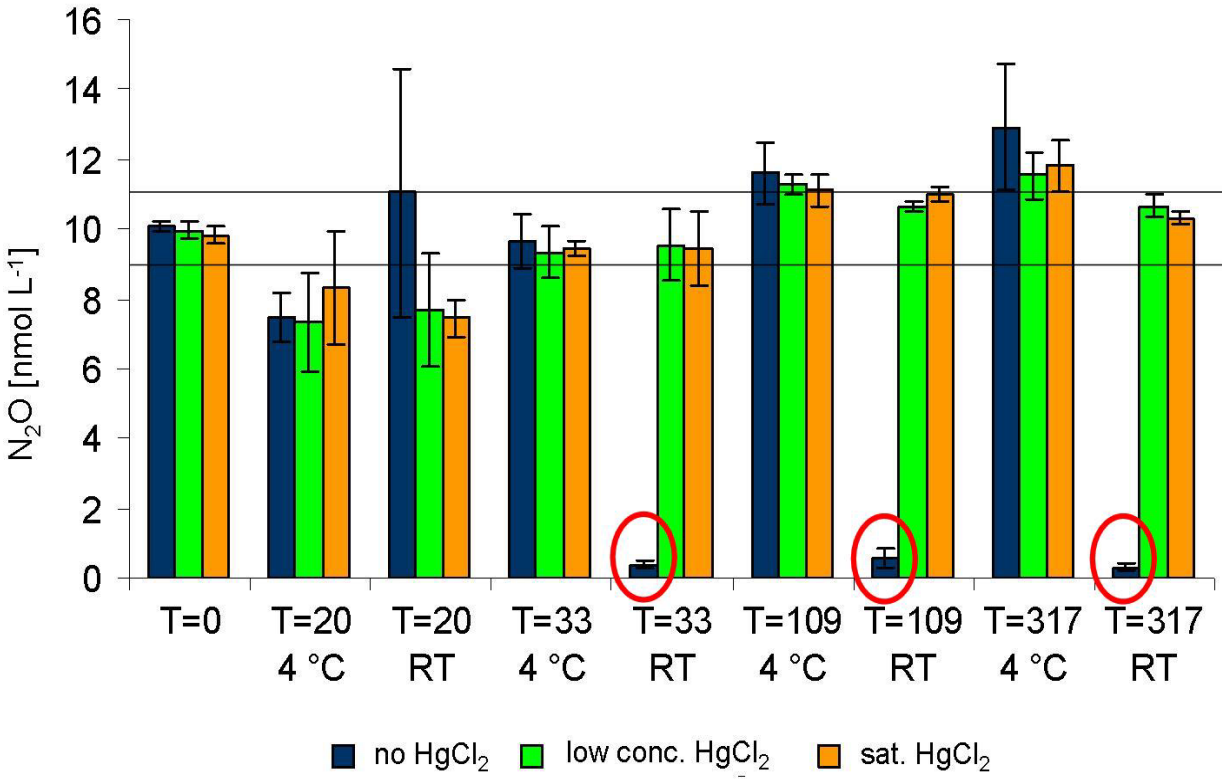

Fig. 1: N_2O concentration after different treatments, lines give an estimated error of ± 1 nmol L^{-1}, results of N_2O measurements of not poisoned samples stored at room temperature are red circled

N_2O concentrations measured directly after sampling (t = 0) showed a mean concentration of 9.97 ± 0.14 nmol L^{-1}, independent from poisoning. These concentrations correspond to the expected equilibrium concentration of 9.63 nmol L^{-1} in surface waters. The equilibrium concentration was calculated with an atmospheric N_2O concentration of 318 ppb, and the ambient salinity and temperature. 10.68 ± 1.07

With one exception, after 10 months we found no significant change in N_2O concentrations either in different poisoned treatments or in treatments stored at different temperatures. The mean N_2O concentration of the treated samples was 10.68 ± 1.07 nmol L^{-1} (samples measured after 20 days and not poisoned samples stored at room temperature were excluded). However, there might be a weak trend to increasing N_2O concentrations in poisoned samples with time; more distinct in cooled samples than in samples stored at room temperature (see Fig. 1).

Only not poisoned samples stored at room temperature showed a distinct decrease in N_2O concentrations after 33 days to approximately zero (see Fig. 1, red circles). The decrease in measured N_2O concentrations after 20 days was probably an artefact due to problems with the carrier gas flow. However, to demonstrate that the decrease of N_2O concentrations of not poisoned samples stored at room temperature does not occur before 20 days of storage these data were also shown.

[*Elkins,* 1980] published results of storage experiments with freshwater from the Boston´s Muddy river, where 50 mL samples were poisoned with 200 µL saturated $HgCl_2$ and stored at 5 to 10 °C. He found no significant storage effect on poisoned samples after 45 days. This is in general agreement with our results. However, based on the fact that *Elkins* [1980] used a freshwater sample with a comparable high N_2O concentration of 591 nmol L^{-1}, these experimental conditions were not representative for oceanic N_2O concentrations.

Yoshinari [1976] tested the effect of $HgCl_2$ on water samples from the open Atlantic Ocean. 650 mL samples of different depths were either poisoned with 0.5 mL of 3% $HgCl_2$ solution or not poisoned. With the exception of water from the oxygen minimum layer he found no differences between both treatments. In the oxygen minimum layer N_2O concentrations in the not poisoned samples were two times higher than in those poisoned with $HgCl_2$. The effect of $HgCl_2$ was also tested by *Kieskamp et al.* [1988]. They found an increase of N_2O concentrations in sediment pore water after addition of $HgCl_2$; assuming a reduction of nitrite to N_2O by Fe^{2+}. However, mechanisms of these reactions are not completely understood.

The distinct decrease of N_2O concentrations in not poisoned samples stored at room temperature indicates a conversion of N_2O by biological activities. Without poisoning a degradation of O_2 by ongoing biological activities can be assumed, which lead to a change to anoxic conditions in the vials. Under anoxic conditions N_2O can be used as an electron acceptor instead of O_2 [*Elkins et al.,* 1978; *Cohen and Gordon*, 1978]. Therefore, the concentrations clearly decrease and remain near zero.

4. Conclusions

- There was no difference between poisoned samples with low concentrated or saturated $HgCl_2$ solution. Thus, it is not necessary to work with saturated $HgCl_2$ solution.
- There is no difference between poisoned samples stored at room temperature or at 4 °C, on condition that samples are poisoned. Thus, samples must not be transported with cooling.
- If samples are measured closely within a few days after sampling, it is not necessary to poison them.

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